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ANAEROBIC TRANSFORMATION PROCESSES:

A REVIEW OF THE MICROBIOLOGICAL LITERATURE

by

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The purpose of this review is to evaluate and summarize available information about the role of microorganisms in the anaerobic transformation of xenobiotic compounds in natural environments. The review focuses on the anaerobic transformation of xenobiotic compounds and natural structural analogs. A discussion of the anaerobic degradation of benzoate is used to introduce the anaerobic microorganisms important to the degradation of xenobiotics in the environment. This is followed by a review of the degradation of several compound classes including hydroxy and methoxy substituted and lignoaromatic compounds, halogenated aromatic and aliphatic compounds, aromatic and aliphatic hydrocarbons, nitrogen containing compounds, and ether linked compounds. A comprehensive list of compounds known to be degraded under anaerobic conditions is presented in tabular form. A brief discussion of invironmental factors that may affect anaerobic degradation processes is presented along with a review of methods important to measuring the rate of disappearance of xenobiotics in environmental samples.

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FOREWORD

As environmental controls become more costly to implement and the penalties for judgment errors become more severe, environmental quality management requires more efficient management tools based on greater knowledge of the environmental phenomena to be managed. As part of this Laboratory's research on the occurrence, movement, transformation, impact and control of environmental contaminants, the Biology Branch conducts research to predict the rate, extent, and products of biological processes that control pollutant fate in soil and water ecosystems.

Techniques for predicting the transport and transformation of toxic chemicals in the environment require information on microbial degradation processes. This report presents a literature review of research into the role of microorganisms in the anaerobic transformation of xenobiotic compounds. In addition to a compilation of all the available data, this report represents an attempt to relate the data from tangential areas to the transformation of xenobiotics in natural environments. Information gathered in the review will be applied to the Laboratory's research to assess kinetic concepts for describing the transformation and/or degradation of toxic substances in aerobic and anaerobic environments.

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ABSTRACT

The purpose of this review is to evaluate and summarize available information about the role of microorganisms in the anaerobic transformation of xenobiotic compounds in natural environments. The review focuses on the anaerobic transformation of xenobiotic compounds and natural structural analogs. A discussion of the anaerobic degradation of benzoate is used to introduce the anaerobic microorganisms important to the degradation of xenobiotics in the environment. This is followed by a review of the degradation of several compound classes including hydroxy and methoxy substituted and lignoaromatic compounds, halogenated aromatic and aliphatic compounds, aromatic and aliphatic hydrocarbons, nitrogen containing compounds, and ether linked compounds. A comprehensive list of compounds known to be degraded under anaerobic conditions is presented in tabular form. A brief discussion of environmental factors that may affect anaerobic degradation processes is presented along with a review of methods important to measuring the rate of disappearance of xenobiotics in environmental samples.

From this review I was able to conclude that several common degradative pathways appear to be shared by all bacteria and bacterial communities capable of anaerobic degradative activity. Ring reduction precedes ring cleavage in the degradation of aromatic compounds. Substitutents of aromatic compounds are generally removed to produce benzoate or phenol before ring reduction and ring cleavage occurs. In most substrates, including sewage sludge, sediments, and aquifer material, degradation results from the activity of microbial consortia having methanogenic and sulfidogenic organisms acting as the final electron acceptors of the community. The removal of ring substituents, ring reduction and ring cleavage is generally performed by fermentative members of the consortia. Despite these advances there is a lack of information on the turnover rates of hazardous organic chemicals in anaerobic environments. Work in this area may, however, be facilitated by the increasing number of aerobic studies directed toward prediction of the persistence and concentration of toxic chemicals in natural environments.

This report covers a period from July 1985 to June 1986, and work was completed as of June 1986.

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REVIEW OF ANAEROBIC TRANSFORMATION PROCESSES

INTRODUCTION

The purpose of this review is to evaluate and summarize available information concerning the role of microorganisms in the anaerobic transformation of xenobiotic compounds in natural environments. Unfortunately, information that relates directly to anaerobic transformation of xenobiotics in the environment is limited. A large body of information, however, does exist concerning the anaerobic transformation (or degradation) of these synthetic chemicals and related natural compounds by pure organisms, mixed cultures, and adapted and unadapted sewage sludge and sediment systems. In addition to a compilation of all the available data, this report represents an attempt to relate the data from tangential areas to the transformation of xenobiotics in natural environments.

To focus this review, I have included only information concerning the anaerobic transformation of xenobiotic compounds and natural structural analogs. Naturally occurring compounds are included because it is generally acknowledged that the closer in structure a xenobiotic compound is to a natural substrate the more likely it will be transformed in the environment. Stanley Dagley (1972) stated it well when he wrote "A man-made compound will be biodegradable only when the relevant microbes are able to use the enzymic apparatus acquired during evolution designed to exploit the diverse sources of energy found in nature." Thus, information on the transformation of many naturally occurring compounds will be valuable in determining the potential for degradation of xenobiotic compounds.

CHARACTERISTICS OF ANAEROBIC MICROORGANISMS

Anaerobic and aerobic degradation of hazardous organic chemicals are markedly different processes. This difference is particularly evident in the degradation of aromatic compounds. Anaerobic degradation requires extensive reduction of the ring nucleus before ring cleavage. Benzoate, for example, is converted to cyclohexanecarboxylate by a six-electron reduction in the initial steps of anaerobic degradation (Sleat and Robinson 1984). Aerobic processes, on the other hand, make direct use of molecular oxygen to hydroxylate the ring before ring cleavage, a process that also requires interaction with molecular oxygen. Benzoate in this case is initially oxidized under aerobic conditions to catechol (Reiner 1971, Reiner 1972), which is subsequently cleaved by the incorporation of molecular oxygen to produce cis,cis-muconic acid (Dagley and Gibson 1965).

Progress toward understanding the biochemical details of the anaerobic degradation of xenobiotics has been slow because of the experimental difficulties attending the use of communities of microorganisms. For the most part, anaerobic degradation of xenobiotic compounds generally requires the concerted effort of several metabolic groups of bacteria. The initial group ferments the parent compound to products such as saturated fatty acids, H_2 and CO_2. An intermediate group, the proton-reducing acetogenic bacteria (Bryant 1976, McInerney et al. 1979, McInerney et al. 1981), converts the long chain fatty acids to acetate, propionate, CO_2, and H_2. The terminal metabolic group includes the many methanogenic species (Balch et al. 1979, Whitman 1985) and possibly sulfur-reducing species (Mountfort and Bryant 1982) that catabolize the acetate produced by the other groups to CO_2 and CH_4, and rapidly use the H_2 produced to reduce CO_2 to methane.

The integrity and efficiency of anaerobic consortia are maintained in part by what has been termed "interspecies hydrogen transfer." This process was first described by Bryant et al. (1967). Fermentative bacteria normally reoxidize their reduced coenzymes by transfer of electrons to an organic terminal electron acceptor such as pyruvate, lactate, acetate, etc. However, in the presence of extremely low concentrations of H_2, some bacteria have the ability to reoxidize their reduced coenzymes by proton reduction. Hydrogen concentrations are maintained at a low level by the H_2-scavenging activity of methanogenic bacteria or some sulfate-reducing bacteria. The proton-reducing partner in such syntrophic associations gains a small energetic increment by H_2 release because cellular intermediates, which would otherwise be reduced for reoxidizing reduced coenzymes, can be further oxidized to acetate. The extremely low H_2 concentrations ($<10^{-4}$ atmosphere) that permit proton reduction have been measured in anaerobic sediments (Winfrey et al. 1977; Strayer and Tiedje 1978; Winfrey and Zeikus 1979; Jones et al. 1982).

Despite the importance of anaerobic consortia, a number of anaerobic organisms have been shown to grow on substituted aromatic compounds as the sole source of carbon. In many cases the substrates are completely mineralized and the presence of acetogenic or hydrogen utilizing bacteria. Such organisms include the purple nonsulfur bacteria such as Rhodopseudomonas palustris and Rhodopseudomonas gelatinosa (Evans 1977), nitrate reducing bacteria such as Pseudomonas (PN1) and Moraxella sp. (N.C.I.B. 11086) (Evans 1977) and, sulfate reducing organisms such as Desulfococcus multivorans, Desulfonema magnum, and Desulfosarsina varaibilis (Widdel 1980).

The relative importance of each mechanism to the degradation of a xenobiotic released to a particular environmental niche will depend on the chemical structure of the xenobiotic and the physical/chemical characteristics of the site. Microorganisms active in the degradation of the xenobiotic will be limited to those organisms that have or can produce enzymes and possibly transport systems that can recognize and act on the xenobiotic. The closer in structure a xenobiotic compound is to the structure of a common natural substrate, the more likely that it will be transformed or degraded by a large variety of microorganisms. These organisms are only active if they are able to grow and compete under the physical/chemical constraints of the particular environment. For example, in estuarine sediments the microbial populations are

generally dominated by sulfate reducing microorganisms. Alternatively, in fresh water sediments where the concentration of oxidized sulfur species is low, methanogenic microorganisms dominate. If the dominating organisms are capable, the xenobiotic has a good chance of being rapidly degraded. Unfortunately, if minor populations, such as methanogenic microorganisms in an estuarine environment, are the active members of the community, the chemical would most likely be degraded at a slower rate.

Benzoate is a natural product and a structural component of a large number of xenobiotic compounds. Benzoate was also one of the first aromatic compounds shown to be degraded anaerobically. Tarvin and Buswell (1934) reported that benzoate and a number of similar aromatic compounds were completely utilized with the production of CO_2 and methane by a sewage sludge inoculum under strictly anaerobic conditions. Since this initial discovery, the degradation of benzoate has been demonstrated in all major environmentally important anaerobic microorganisms. The following summary of anaerobic benzoate metabolism will be used to introduce these microorganisms.

Photosynthetic bacteria

Scher (1960) and Procter and Scher (1960) were the first to demonstrate anaerobic photometabolism of benzoic acid by showing that Rhodopseudomonas palustris could grow on benzoate anaerobically in the light. In addition, cells grown anaerobically on a mineral salts-benzoate medium in the light was shown, by monometric techniques, to oxidize benzoate, protocatechuate, catachol, and α-oxoglutarate in the dark. Unfortunately, these results led the authors to incorrectly place catechol as an intermediate in the metabolic pathway. This conclusion apparently resulted from a literal interpretation of van Niel's general metabolic schemes (van Niel 1941) for photosynthetic bacteria and plants. One of the products of the light reaction was considered to be a strong oxidant ["OH"], which in plants was converted to oxygen and in bacteria was used to oxidize substrates. Thus, in this early work, the light-induced oxidant and molecular oxygen were considered equivalent and the anaerobic pathway was considered to be the same as the pathway reported for aerobic degradation mechanisms. These experiments, however, could not be repeated. Subsequently, Dutton and Evans (1968) were able to show that R. palustris grown photosynthetically on benzoate was not able to respire in the presence of benzoates or 3-hydroxybenzoate under aerobic conditions. Later they showed (Dutton and Evans 1969) that return to anaerobic conditions in the light permits benzoate utilization to proceed again.

The dependence on anaerobic conditions strongly suggested that anaerobic photometabolism of benzoate was a reductive process. Guyer and Hegeman (1969) concluded this from mutant studies with R. palustris, and suggested that cyclohexanecarboxylate and cyclohex-1-enecarboxylate might be metabolic intermediates. At the same time, Dutton and Evans (1968, 1969), using ^{14}C-labelled benzoate, determined the key intermediates of anaerobic photometabolism of benzoate in R. palustris. Degradation proceeded from benzoate to pimelic acid with the intermediate formation of cyclohex-1-enecarboxylate, 2-hydroxycyclohexanecarboxylate and 2-oxocyclohexanecarboxylate. Pimelic acid was further degraded by β-oxidation.

Evans (1977) postulated that benzoate might be activated to benzoyl phosphate before ring reduction could occur. Benzoyl phosphate, but not benzoyl-CoA, was reduced by a washed chromatophore suspension. Conflicting results have been reported by Whittle et al. (1976) who showed that extracts from R. palustris cells grown photosynthetically on benzoate are capable of the thioesterification of benzoate to form benzoyl-CoA. The cofactors for this reaction were ATP and Mg^{2+}. Furthermore, Whittle et al. (1976) obtained a cell-free system capable of converting cyclohex-1-enecarboxylate to pimelate. These reactions required the addition of CoA, ATP, Mg^{2+}, and NAD^{+}. More recently, Hutber and Ribbons (1983) found that anaerobic cultures of R. palustris produced a benzoyl-CoA synthetase activity in response to benzoate. A similar CoA synthetase could be induced by cyclohexane carboxylate under aerobic conditions. The enzymes necessary for β-oxidation of cyclohexane carboxyl-CoA appeared to be constitutive in both aerobic and anaerobic cultures. Harwood and Gibson (1986) have shown that the uptake of benzoate by R. palustris is not the result of either passive diffusion or active transport, but that accumulation of benzoate is closely coupled to the formation of benzoyl-CoA. The formation of benzoyl phosphate could not be ruled out as the rate limiting step in the uptake of benzoate. These most recent findings provide strong circumstantial evidence that the substrate for ring reduction and subsequent cleavage, however, is benzoyl-Co-A.

A metabolic scheme for the anaerobic photometabolism is shown in Fig. 1. The scheme, which incorporates the work of Whittle et al. (1976), Hutber and Ribbons (1983), and Harwood and Gibson (1986), is essentially an extension of the degradative pathway first described by Dutton and Evans (1969). The conversion of cyclohexanecarboxyl-CoA to cyclohex-1-enecarboxyl-CoA has not been demonstrated in crude cell extracts and is included here because of the known flavoprotein character of the α,β-acyldehydrogenases of fatty acid β-oxidation. If the benzoyl-CoA reduction mechanism could go directly to the cyclohex-1-enecarboxyl-CoA (dotted line, Fig. 1), the proposed dehydrogenation step would be redundant as a major part of the pathway. The conversion of 2-oxocyclohexanecarboxyl-CoA to pimelyl di-CoA has been included because it is consistent with fatty acid β-oxidation. Because the reaction leads to ring cleavage, both CoA groups are on the same dicarboxylic acid.

The ecological significance of anaerobic photometabolism of xenobiotic compounds is unclear. Rhodospirillaceae occur in all aquatic environments (Pfennig 1967) and in soil, where their numbers increase with increasing moisture content (Pfennig and Truper 1973), so they could play a significant role in the dissimilation of xenobiotics in these environments. Unfortunately, their photometabolic activity is limited to those areas where anaerobic and euphotic zones coincide, such as small eutrophic lakes during summer stratification, flooded soils, and anaerobic surface sediments in ponds and ditches.

Anaerobic respiring bacteria

The potential for anaerobic degradation of benzoate and other aromatic compounds by anaerobic respiration with nitrate as the terminal electron acceptor exists in a large number of environments. Bacteria capable of nitrate

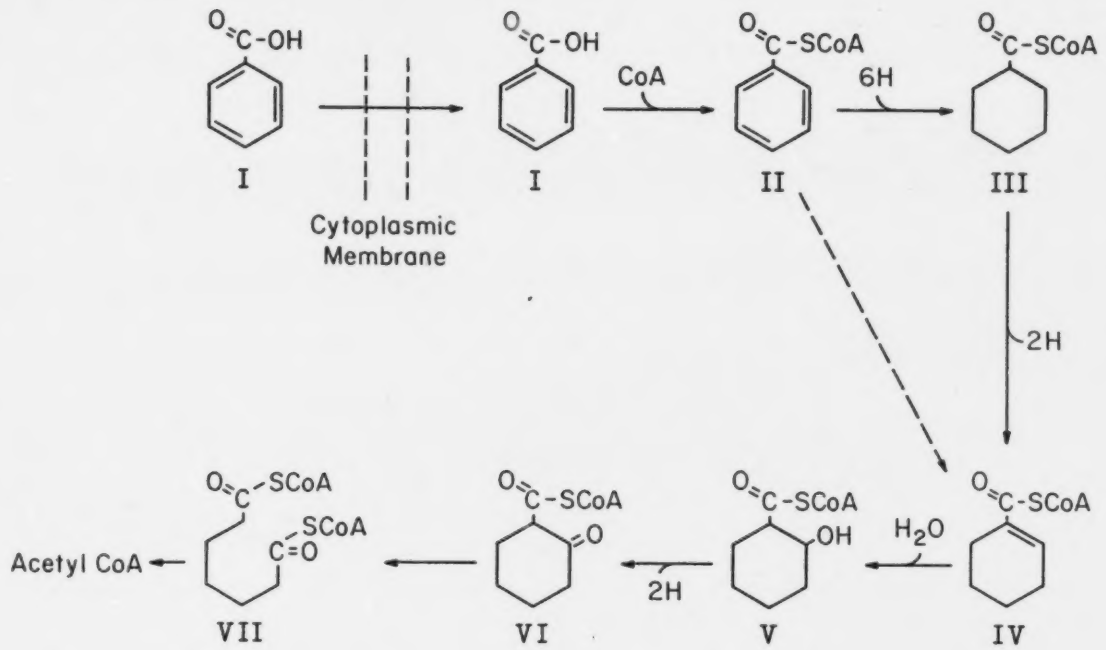

Fig. 1. Proposed pathway for the anaerobic photometabolism of benzoic acid. Compounds are: (I) benzoic acid, (II) benzoyl-CoA, (III) cyclohexane-carboxyl-CoA, (IV) cyclohex-1-enecarboxyl-CoA, (V) 2-hydroxycyclohexane-carboxyl-CoA, (VI) 2-oxocyclohexanecarboxyl-CoA, (VII) pimelyl di-CoA.

reduction have been found in all types of soil, in activated sludge, and in marine and freshwater sediments (Jeter and Ingraham 1981).

Williams and Evans (1973) isolated a soil microorganism able to grow anaerobically on benzoate in the presence of nitrate. Isotope-dilution experiments in conjunction with thin layer chromatography and gas chromatography suggested that a reductive mechanism of ring metabolism, similar to that shown in R. palustris by Dutton and Evans (1969), was operating. In more recent work this isolate was identified as a Moraxella sp. (Williams and Evans 1975). Additional intermediates in the ring cleavage reactions were identified, and, although they were not identical with those for photometabolism, the basic principle of ring reduction preceding ring cleavage under anaerobic conditions was confirmed.

The degradative pathway of benzoate metabolism coupled to nitrate respiration, as proposed by Evans (1977), is shown in Fig. 2. This pathway diverges from the scheme for R. palustris at 2-oxocyclohexanecarboxylate. For R. palustris a hydrolytic cleavage occurs to give pimelic acid whereas decarboxylation followed by ring cleavage gives adipic acid for nitrate respiring organisms. Coenzyme-A is proposed as a cofactor in the conversion of cylohexanecarboxylate to the 2-oxo-derivative; however, no direct evidence has been reported to support this.

Interestingly, in some aerobic sediments both oxygen-mediated and nitrate-dependent aromatic metabolism may occur simultaneously or in concert. Focht and Verstraete (1977) considered anoxic microzones, such as those within particles, as possible sites of denitrification in aerobic sediments, and Jones (1979) has suggested that similar micro-environments were responsible for nitrate reductase activity in aerobic freshwater sediments. Fabig et al. (1980) observed that, in the presence of trace amounts of oxygen, benzoate degradation and nitrate reduction occurred simultaneously in either an enriched culture or a pure culture of P. aeruginosa. Both cultures were capable of ortho-ring fission. On the other hand, Fabig et al. (1980) found that denitrifying bacteria, such as a Acinetobacter sp., and a Moraxella sp., that did not have oxygenase activity were unable to use benzoate under microaerophilic conditions. The authors speculated that under "anaerobic" conditions trace amounts of oxygen were required for ring cleavage, and subsequently the aliphatic products were metabolized by nitrate respiration. Similar results were observed by Sleat (1981) in studies of the growth of nitrate respiring facultative anaerobes capable of aerobic but not anaerobic utilization of p-hydroxybenzoate. He found that growth of the organisms on p-hydroxybenzoate was increased by the presence of nitrate when oxygen was supplied at trace levels.

The number of obligately anaerobic microorganisms reported to grow on benzoate or other aromatic compounds in the presence of inorganic terminal electron acceptors is limited. A few sulfate reducing bacteria have been reported to utilize benzoate (Widdel 1980). Organisms from three distinct bacterial genera, Desulfococcus, Desulfonema and Desulfosarcina, able to metabolize benzoate with sulfate as the electron acceptor were isolated. However, no information on the degradative pathways has been reported.

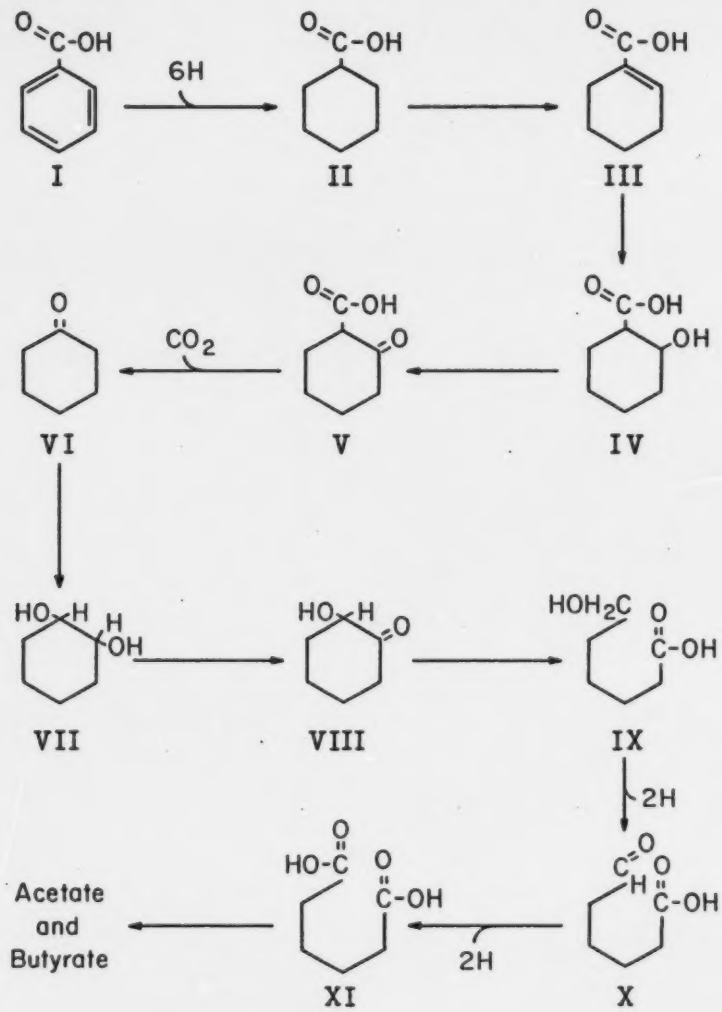

Fig. 2. Proposed pathway for the anaerobic degradation of benzoic acid by nitrate respiring organisms. Compounds are: (I) benzoic acid, (II) cyclohexanecarboxylic acid, (III) cyclohex-1-enecarboxylic acid, (IV) 2-hydroxycyclohexanecarboxylic acid, (V) 2-oxocyclohexanecarboxylic acid, (VI) cyclohexanone, (VII) 1,2-dihydroxycyclohexane, (VIII) 2-hydroxycyclohexanone, (IX) 6-hydroxyhexanoic acid, (X) adipic acid semialdehyde, (XI) adipic acid.

Methane-producing consortia

Anaerobic fermentation of xenobiotic compounds generally requires the concerted effort of several metabolic groups of bacteria to degrade a compound to CO_2 and CH_4. The initial group ferments the parent compound to products such as saturated fatty acids, H_2, and CO_2.

A variety of bacterial consortia have been described and a number of these have been shown to anaerobically degrade benzoate and other aromatic compounds (Tarvin and Buswell 1934, Clark and Fina 1952, Fina and Fiskin 1960, Nottingham and Hungate 1969, Ferry and Wolfe 1976, Shlomi et al. 1978). Attempts to isolate component members of the consortia have produced a number of methane-producing and nonmethanogenic bacteria.

Sufficient evidence exists to suggest that both autotrophic and acetoclastic methanogens function in benzoate degrading consortia. Ferry and Wolfe (1976) isolated Methanobacterium formicicum and Methanospirillum hungatei from anaerobic benzoate enrichments but were unable to isolate an acetate utilizing methanogen. Methanobrevibacterium ruminantium and Methanospirillum hungatei were tentatively identified in enrichments by Balba and Evans (1977). Unfortunately, only circumstantial evidence supports a role for acetate utilizing methanogens in benzoate degrading consortia. Shlomi et al. (1978) identified a gram-negative rod that formed long chains, in a benzoate-methanogenic enrichment. Healy et al. (1980) observed similar long chains of rods in a ferulic acid enrichment. The organisms were thought to be identical to the acetate utilizing methanogen Methanobacterium soehngenii (Zehnder et al. 1980, Huser et al. 1982). The organism forms very long flexible filaments that tend to aggregate in bundles. Microscopic observations revealed the presence of characteristic bundles in the methanogenic-benzoate enrichments of Sleat and Robinson (1983). Methanothrix soehngenii can use acetate but not methanol, methylamine, formate or H_2-CO_2 for growth and methane production (Huser et al. 1982).

The isolation of nonmethanogenic organisms from stable consortia has had limited success. Ferry and Wolfe (1976) isolated a bacterium similar in appearance to Pseudomonas PN-1 (Taylor et al. 1970) that grew aerobically but not anaerobically on benzoate. A facultative, gram-negative bacterium that grew aerobically on p-hydroxybenzoate and anaerobically on benzoate in the presence of nitrate was isolated by Balba and Evans (1977). Attempts to reconstitute a benzoate-methanogenic consortium with M. hungatei were unsuccessful (Balba 1978). Abram and Nedwell (1978a) were somewhat more successful in reconstituting a benzoate degrading consortium. They found that recombination of a gram-negative pleomorphic rod and a Methanococcus sp., both isolated from the same benzoate enrichment, resulted in methanogenesis from benzoate in 3 of 20 tries.

McInerney et al. (1979, 1981) and Boone and Bryant (1980) have described the isolation of aliphatic acid metabolizing bacteria in syntrophic association with either sulfate reducing or methanogenic bacteria. To isolate a bacterium in coculture with H_2 utilizing organisms, aliquots of serial 10-fold dilutions of enrichments were transferred to roll tubes containing 4 ml of a basal growth medium plus 0.5 ml of an actively growing culture of the H_2 utilizing organism.

The basal growth medium did not support growth of the H_2-utilizing organism or the organism to be cocultured. Positive cultures generally contained a synthroph that degraded aliphatic acids to H_2, CO_2 and acetate in addition to the H_2 utilizing bacterium. Mountfort and Bryant (1982) have used this technique to isolate a benzoate catabolizing, gram negative, nonsporing, rod-shaped bacterium that grows in anaerobic syntrophic association with hydrogen utilizing Desulfovibrio sp. or with Methanospirillum hungatei. More recently, Barik et al. (1985) were able to isolate two bacterial strains, P-2 and PA-1, that could grow syntrophically with Wollinella succinogens on phenol and phenylacetate, respectively.

Evans (1969) has reported that Soehngen observed methane production from aromatic compounds as early as 1906. The earliest published report appears to be that of Tarvin and Buswell (1934). They demonstrated that a range of aromatics were metabolized to methane and carbon dioxide with an efficiency of greater than 90%. Many subsequent studies emphasize the biochemical mechanisms of anaerobic aromatic metabolism. Although ring reduction preceded ring cleavage, the reduction intermediates varied depending on the enrichment. The metabolic scheme for benzoate degradation by methanogenic consortia is shown in Fig. 3. The scheme is a consolidation of published results from a number of laboratories. Shlomi et al. (1978) described a consortium from enrichments prepared from black mud from a small polluted river that degrades benzoate by the reductive pathway reported for R. palustris (Dutton and Evans 1969). Intermediates consistent with the reductive pathway, trans-2-hydroxycyclohexanecarboxylate, 2-oxo-cyclohexanecarboxylate, pimelate, caproate, butyrate, and acetate were identified from pyridine extracts of freeze-dried samples of actively growing cultures. In a separate study, Balba and Evans (1977), using isotope trapping techniques, detected the formation of adipic acid from benzoate in methanogenic cultures obtained from sewage sludge. The presence of adipate was indicative of decarboxylation before ring cleavage as found for the nitrate respiring Moraxella sp. investigated by Williams and Evans (1975). Keith et al. (1978) proposed that benzoate is reduced step-wise to cyclohexanecarboxylate followed by a reductive cleavage of the C_1 - C_2 bond to give heptanoate. Heptanoate would be degraded to propionate by β-oxidation. They identified cyclohexanecarboxylate, cyclohex-1-enecarboxylate, heptanoate, valerate, butyrate, propionate and acetate in benzoate-methanogenic cultures.

Grbic-Galic and Young (1985) isolated a number of intermediates from benzoate degrading methanogenic consortia treated with bromoethanesulfonic acid (BES) to inhibit methane formation. The major intermediates isolated included cyclohexanecarboxylate, cyclohexanone, 1-methylcyclohexanone, pimelate, adipate and heptanoate. The identification of pimelate is consistent with the photosynthetic pathway (Evans 1977); the intermediates, cyclohexanone and adipate, are consistent with decarboxylation before ring cleavage as reported for nitrate respiring organisms (Evans 1977). The formation of heptanoate was suggested to result from ring cleavage of 2-methylcyclohexanone in contrast to the reductive cleavage of cyclohexanecarboxylate proposed by Keith et al. (1978). Direct confirmation of either mechanism will await isolation and charaterization of the appropriate enzyme systems.

Not surprisingly, several unusual reduced compounds (toluene, benzene, cyclohexane, and methylcyclohexane) were detected in BES-inhibited consortia and nonmethanogenic mixed cultures derived from the inhibited consortia. It was suggested that these compounds were formed when the normal flow of electrons and hydrogen was interrupted with BES. For similar complex systems (Wolin 1974; Wolin 1976; Bryant 1979), in the absence of methanogenesis, reduced products such as ethanol, propionate, butyrate, lactate, succinate, or adipate are formed. It is interesting to speculate that some of the variability in intermediates (Fig. 3) observed for uninhibited methanogenic consortia may result from a decrease in normal level of "interspecies hydrogen transfer," which modulates the efficient conversion of organic compounds to CO_2 and CH_4.

Methanogens and sulfate reducing bacteria have been detected in a number of ecosystems that include mud, sediment, subsurface materials and flooded soils of both marine and freshwater environments (Zeikus 1977). Methanogenic activity dominates in environments (freshwater) having low concentrations of oxidized sulfur species and, conversely, sulfate reducing activity dominates in environments (marine) that contain high concentrations of oxidized sulfur species. Sulfate reducing bacteria have been shown to outcompete methanogenic bacteria in the presence of sulfate (Abram and Nedwell 1978a,b; Lovley and Klug 1982; Winfrey and Zeikus 1977). Sleat and Robinson (1983) examined the methanogenic fermentation of benzoate in anaerobic freshwater mud and showed that carbon dioxide was the only significant end-product of benzoate fermentation in the presence of sulfate, whereas in the absence of sulfate both methane and carbon dioxide were formed. The apparent inhibition of methanogenesis by sulfate reducing bacteria has been attributed to thermodynamic principles (Winfrey and Zeikus 1977, Zehnder 1978) or kinetic competition for substrates (Abram and Nedwell 1978, Kristjansson et al. 1982, Schonheit et al. 1982, Winfrey and Zeikus 1977). Lovley (1985) has reemphasized that sulfate-reducing bacteria may outcompete methanogenic bacteria by maintaining the hydrogen partial pressure below a minimum threshold necessary for methane production (Lovley et al. 1982). He recently demonstrated that methanogenic isolates cannot consume hydrogen below a partial pressure of 6.5 Pa. In contrast, in the absence of sulfate some sulfate-reducing bacteria (Fiebig and Gottschalk 1983, Traore et al. 1983, Phelps et al. 1985) are acetogenic proton reducers. Thus, in the absence of sulfate, they function syntrophically and produce substrates for methanogenic bacteria.

Autotrophic acetogens

A fourth class of organisms known to utilize H_2 in anaerobic environments is the acetogenic bacteria (Ljungdahl and Wood 1982). Unlike the methanogenic and sulfate reducing bacteria that utilize both H_2 and acetate, the major carbon intermediate, the acetogens utilize H_2 and produce acetate from CO_2. Braun et al. (1979) reported viable counts of acetogens to be ca. 1% of those of methanogens in sludge and lake sediments. It also has been estimated (Lovley and Klug 1983) that acetogens account for ca. 5% of H_2 consumption in the sediments of hypereutrophic Wintergreen Lake. In the profundal sediments of Blelham Tarn (English Lake District), where anaerobic degradation processes can be limited by the input of organic carbon, hydrogen consumption by acetogens can be as high as 50% of the methanogenic rate (Jones and Simon 1985). Apparently

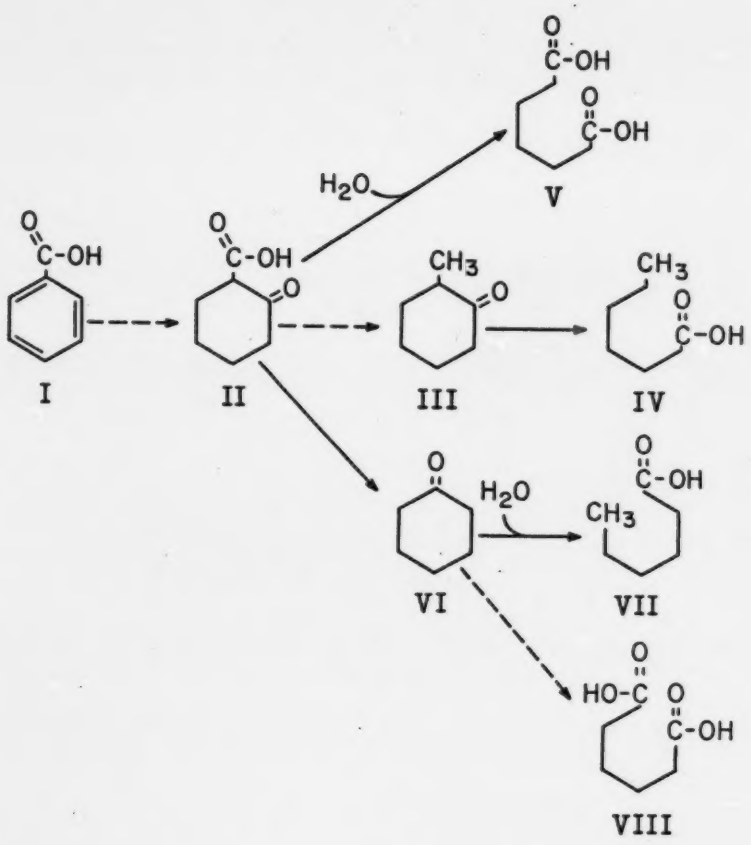

Fig. 3. Proposed pathway for the degradation of benzoic acid by methanogenic consortia. Compounds are: (I) benzoic acid, (II) 2-oxocyclohexanecarboxylic acid (III) 2-methylcyclohexanone, (IV) heptanoic acid, (V) pimelic acid, (VI) cyclohexanone, (VII) caproic acid, (VIII) adipic acid.

the acetogenic bacteria may have a larger role in the mesotrophic to oligotrophic lakes as opposed to the more eutrophic lakes.

Although acetogens have not been demonstrated to function in the degradation of benzoate, other than acting as a hydrogen sink, acetogens have been shown to demethylate methoxylated aromatic compounds to yield the hydroxy derivative, carbon dioxide and acetic acid. Using anaerobic enrichments with methoxylated aromatic compounds as substrates, Bache and Pfennig (1981) isolated two bacterial strains, NZva16 and NZva24, which were recognized as Acetobacterium woodii. Both isolates and the type strain of Acetobacterium strains were able to grow with aromatic compounds containing methoxyl groups. Product analysis revealed that the aromatic ring was not cleaved and that the corresponding hydroxy derivatives of the methoxylated compounds were formed. For example, vanillic acid was transformed to protocatechuic acid and syringic acid was converted to gallic acid. Recently, a novel anaerobic gram-negative rod bacterium that utilized o-methyl substituents of monoaromatic acids as a sole source of carbon was isolated from municipal sewage sludge by Frazer and Young (1985). The organism transformed syringic acid to gallic acid, vanillic acid to protocatechuic acid, and ferulic acid to hydrocaffeic acid. Frazer and Young (1986) postulated that the carboxyl carbon of the product acetate is derived from CO_2 and the methyl carbon is derived from the o-methyl substituent of vanillate. In support of this hypothesis they showed that, for every o-methyl carbon of vanillic acid converted to acetate, two were converted to carbon dioxide.

ANAEROBIC DEGRADATION OF SELECTED COMPOUND CLASSES

Progress in understanding the anaerobic degradation of xenobiotic compounds has been slow primarily because of the experimental difficulties in using bacterial consortia that are extremely sensitive to oxygen. However, in recent years, a number of aromatic and aliphatic compounds have been shown to be degraded by anaerobic microorganisms and consortia. For example, the lignoaromatic compounds--ferulic acid, vanillin, cinnamic acid, protocatechuic acid, and catechol--can be converted to methane by anaerobic bacterial consortia (Healy and Young 1978, 1979). Suflita et al. (1982) demonstrated that anaerobic microorganisms in lake sediments and sewage sludge degrade halogen-substituted benzoic acid by eliminating halide ion to produce benzoate, which can then be metabolized to methane. Anaerobic dehalogenation may be more important than the corresponding aerobic process. Recent work by Grbic-Galic and Vogel (1986) has shown that benzene and toluene can be degraded to methane and carbon dioxide by a ferulic-acid-adapted anaerobic enrichment. These compounds were generally believed to be resistant to microbial attack in the absence of oxygen.

A comprehensive list of compounds known to be transformed or degraded under anaerobic conditions is presented in Table 1. The table provides a list of references for each compound, the source of the microorganisms for the individual studies, and an indication of the analytical methodology used to measure degradation or transformation. The remainder of this section will be concerned with the anaerobic degradation and transformation of a number of compound classes.

Hydroxy and methoxy substituted and lignoaromatic compounds

Numerous reports have appeared concerning the degradation of hydroxy- and methoxy-substituted benzenes and lignoaromatic compounds. Chemielowski and coworkers described the methanogenic fermentation of phenol in 1964. Since that time Bakker (1977) has demonstrated the anaerobic degradation of phenol to CO_2 in the presence of high concentrations of nitrate. Healy and Young (1978) and Balba and Evans (1979) confirmed the anaerobic degradation of phenol to CO_2 and methane by methanogenic consortia; Healy and Young (1978) extended their studies to demonstrate the degradation of catechol as well. Subsequently, Healy and Young (1979) investigated the anaerobic degradation of eleven aromatic compounds, which included vanillin, vanillic acid, ferulic acid, cinnamic acid, benzoic acid, catechol, protocatechuic acid, phenol, p-hydroxybenzoic acid, syringic acid, and syringaldehyde. Methanogenic populations acclimated to a particular aromatic substrate were simultaneously acclimated to some, but not all, of the aromatic substrates investigated. Boyd et al. (1983) investigated the anaerobic degradation of phenol and the ortho, meta, and para isomers of methoxyphenol and methylphenol (cresol) initially at 50 ppm in diluted municipal sewage sludge. Only o-cresol was not degraded to CO_2 and methane by the end of the 8-week incubation period. The degradation of phenol and seven alkyl-phenols (o-, m-, and p-cresol, 2,5-, 2,6-, 3,4-, and 3,5-dimethylphenol) at concentrations ranging from 100 to 2000 ppm was examined (Fedorak and Hrudey 1984) in anaerobic domestic sludge. Only the lower concentrations of phenol (500 ppm) and p-cresol (200 and 400 ppm) were degraded to methane. At 500 ppm for 2,5-, 3,4-, and 3,5-dimethylphenol, the rate and amount of methane produced was below unamended sludge samples. Phenol did not inhibit methane production until concentrations reached 2000 ppm or more.

Ehrlich et al. 1982 have examined the anaerobic degradation of phenolic compounds contaminating an aquifer at St. Louis Park, Minnesota. Analysis of the in situ concentration of phenols in a series of wells indicated that the concentration of the phenolic compounds was decreasing faster than could be accounted for by dispersion. Sorption of the phenolic compounds was negligible; thus anaerobic degradation was considered the controling factor. The key role of anaerobic bacterial activity was supported by the observations that methane was only found in water samples from the contaminated zone, that methane-producing bacteria were only found in waters from the contaminated zone, and that methane was produced in laboratory cultures of contaminated water inoculated with bacteria from the contaminated zone.

The metabolic pathways for the anaerobic degradation of hydroxybenzenes and methoxybenzenes as well as lignoaromatic compounds can be roughly divided into two groups. One group requires the removal of hydroxy and methoxy groups before reduction and cleavage of the ring and the second group does not. The anaerobic pathways for the degradation of phenol (Bakker 1977, Balba and Evans 1979), ferulic acid (Healy et al. 1980, Grbic-Galic and Young 1985), and coniferyl alcohol (Grbic-Galic 1983) are well documented and have been shown to involve dehydroxylation and demethoxylation of the aromatic ring before reduction and ring cleavage. In contrast, phloroglucinol is reduced to dihydrophloroglucinol without dehydroxylation before 2-oxo-4-hydroxyadipate is formed from ring fission (Whittle et al. 1976).

Bakker (1977) has suggested that phenol is reduced to cyclohexanone followed by hydrolytic conversion of the ring to give n-caproic acid, which can then undergo β-oxidation to yield acetate (Fig. 4). As evidence for this pathway, Bakker pointed to the identification of trace concentrations of caproate in mixed bacterial cultures that were able to convert (U-^{14}C) phenol to $^{14}CO_2$ and radioactive cell material in a nitrate-mineral salts medium. No methane was formed, presumably because of the presence of high nitrate concentrations. Bakker's pathway was later partially confirmed by Balba and Evans (1979), who were able to isolate the metabolic intermediates cyclohexanone, adipate, succinate, propionate, and acetate from a phenol-adapted methanogenic fermentation. In this case the identification of adipate suggests an alternative pathway (Fig. 4) for the cleavage of cyclohexanone, which could be similar to the pathway observed for the nitrate-respiring Moraxella sp. (Fig.2).

The degradation of the lignoaromatic compound, ferulic acid, has been shown to occur anaerobically by a reductive pathway (Fig. 5) that overlaps the previously reported pathways for benzoic acid (Fig. 3) and phenol (Fig. 4). Evidence in support of this pathway has been provided by Healy et al. (1980), Grbic-Galic and Young (1985), and Grbic-Galic (1985). Healy et al. (1980) observed that ferulic acid was biodegraded to CO_2 and methane under strictly anaerobic conditions by a bacterial consortium similar to that observed for benzoate (Shlomi et al. 1978). By incubating the cultures with BES to inhibit methanogenesis, intermediates of phenylacetate, cinnamate, 3-phenylpropionate, benzoate, cyclohexanecarboxylate, adipate, and pimelate were detected. A reductive catabolic pathway was described that, in part, supports the pathway shown in Fig. 5. The pathway merged with the benzoate pathway suggesting that a portion of the pathway may be common to the degradation of many ligoaromatic compounds. In a subsequent study (Grbic-Galic and Young 1985), two additional intermediates, caffeate and p-hydroxycinnamate, were identified in BES-inhibited, ferulic-acid-adapted consortia; the intermediates cyclohexanone, methylcyclohexanone, and isocaproate were identified in BES-inhibited, benzoic-acid-adapted consortia. These results complimented the previous results of Healy et al. (1980), and the combined efforts are responsible for the degradative pathway shown in Fig. 5.

In further investigations of the degradation of ferulic acid, Grbic-Galic (1985) isolated a facultatively anaerobic, gram-negative, nonsporeforming, motile, rod-shaped bacterium from a bacterial consortium degrading ferulic acid. The organism degrades ferulic acid completely under aerobic conditions and partially transforms it under strictly anaerobic conditions, without the addition of exogenous electron acceptors. Anaerobically, ferulic acid was demethoxylated and dehydroxylated with subsequent side chain reduction to yield phenylpropionate and phenylacetic acid. All intermediates were members of the proposed degradative pathway for ferulate (Fig. 5).

As further evidence for the commonalty of these pathways Grbic-Galic (1983) has suggested that the degradation of coniferyl alcohol overlaps with the ferulic acid pathway (Fig. 5). Coniferyl alcohol, was shown to be completely biodegraded to carbon dioxide and methane under strictly anaerobic conditions and ferulic acid and phenylpropionic acid were identified in active cultures. Similarly, results of Taylor (1983) reasonably support a degradative

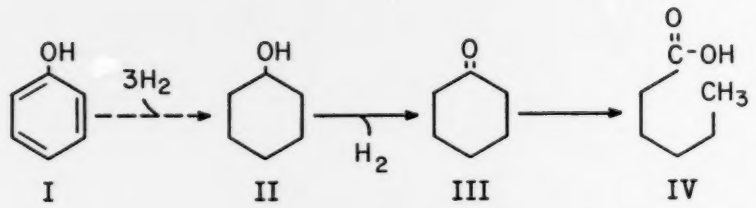

Fig. 4. Proposed pathway for the degradation of phenol by a bacterial consortium in the presence of nitrate. Compounds are: (I) phenol, (II) cyclohexanol, (III) cylohexanone, (IV) caproic acid.

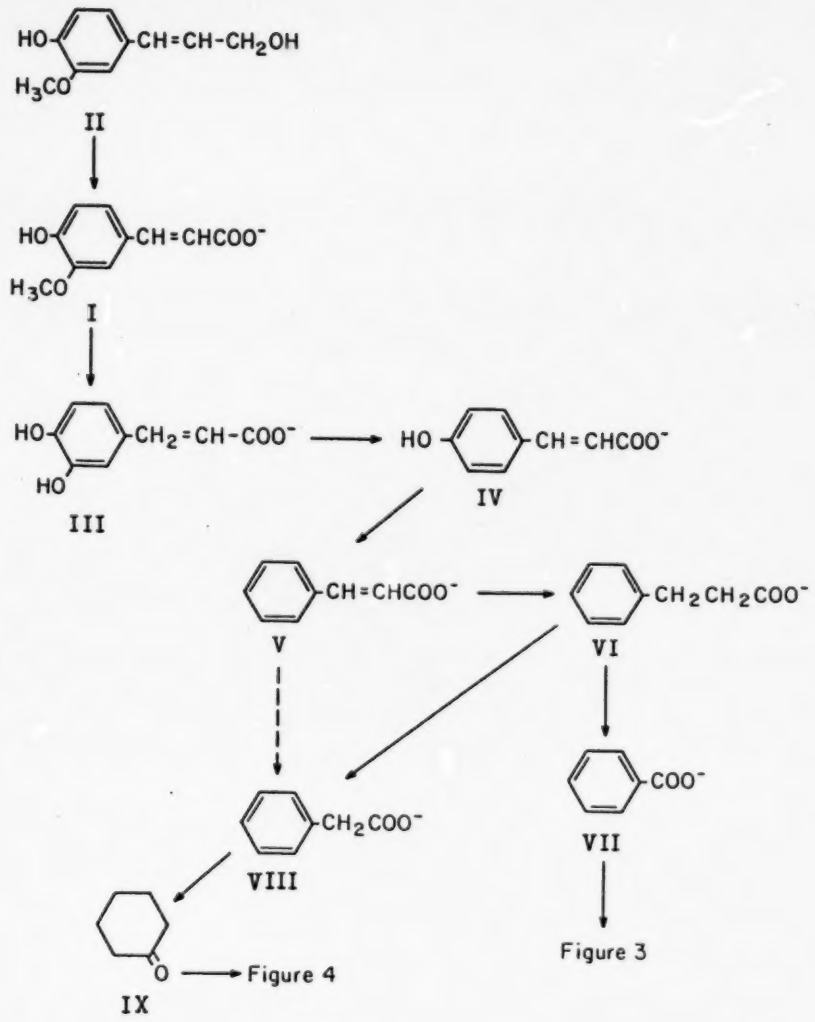

Fig. 5. Proposed pathway for the degradation of ferulic acid and coniferyl alcohol by a methanogenic consortium. Compounds are: (I) ferulic acid, (II) coniferyl alcohol, (III) caffeic acid, (IV) p-hydroxycinnamic acid, (V) cinnamic acid, (VI) phenylpropionic acid, (VII) benzoic acid, (VIII) phenylacetic acid, (IX) cyclohexanone.

pathway for vanillic acid that involves sequential elimination of substituents to generate benzoate, which could then be anaerobically degraded by the reductive pathway for aromatic metabolism. Taylor found that vanillic acid supported the growth of Pseudomonas sp. strain PN-1 when nitrate was used as the electron acceptor. Protocatechuate, m-hydroxybenzoate, p-hydroxybenzoate, and benzoate were immediately metabolized by such cultures, which suggested that they were intermediates in the degradation of vanillic acid.

Some hydroxybenzene and methoxybenzene derivatives may be anaerobically degraded by alternative methods. Kaiser and Hanselmann (1982) prepared an enriched bacterial consortium that degraded syringic acid to carbon dioxide and methane. The authors proposed the degradative pathway shown in Fig. 6. Gallic acid and pyrogallol were rapidly degraded by active cultures and 3,4-dihydroxy-5-methoxybenzoic acid was identified in well-grown cultures. The authors postulated that syringic acid was demethylated and decarboxylated to pyrogallol, which was then reduced by a mechanism analogous to benzoate degradation (Evans 1977). No evidence was presented for the reductive pathway. Strains of Streptococcus bovis and Coprococcus isolated from bovine rumen that were shown (Tsai and Jones 1975, Patel et al. 1981) to anaerobically degrade phloroglucinol to acetate were unable to metabolize benzoate. Schink and Pfennig (1982) isolated five Pelobacter acidigallici strains capable of metabolizing a variety of trihydroxybenzenes to acetate. These strains, which were isolated from both marine and freshwater sediments, produced acetate stoichiometrically from gallic acid, pyrogallol, 2,4,6-trihydroxybenzoate and phloroglucinol. They also converted syringic acid to acetate in co-culture with Acetobacterium woodii. None of the strains were able to metabolize benzoic acid or cyclohexanecarboxylic acid. Whittle et al. (1976) reported that Rhodopseudomonas gelatinosa is able to grow anaerobically in the light with phloroglucinol as the sole source of carbon. Cell-free extracts of the organism were able to reduce phloroglucinol to dihydrophloroglucinol in the dark. The cell-free systems contained cysteine and NADPH(H^+) as reductants. Reactions beyond this point were unclear; however, mass spectral data (Dutton and Evans 1978) did indicate the intermediate formation of 2-oxo-4-hydroxyadipate. These results are summarized in the tentative pathway shown in Fig. 7. Apparently reduction with subsequent ring cleavage can occur for some hydroxylated and methoxylated lignoaromatic compounds.

Halogenated aromatic compounds

Reductive dehalogenation appears to be the general mechanism for initiating the degradation of a number of chloro-, bromo- and iodo-substituted aromatic compounds in anaerobic environments. Ide et al. (1972) and Murthy et al. (1979) observed that, in flooded soils, pentachlorophenol was converted to a number of lesser substituted phenolic products. Reductive dechlorination of a tetrachlorophthalamic moiety was shown by Kirkpatrick et al. (1981) to be the initial step in the degradation of Techlofthalam [N-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorothalamic acid] in flooded rice paddy soil stored in laboratory flasks. Boyd et al. (1983) examined the degradation of ortho-, meta-, and para-chlorophenol in dilute anaerobic sewage sludge. Only p-chlorophenol was not significantly degraded during an 8-week incubation period; o-chlorophenol was metabolized to phenol, which indicated that reductive dechlorination was the initial

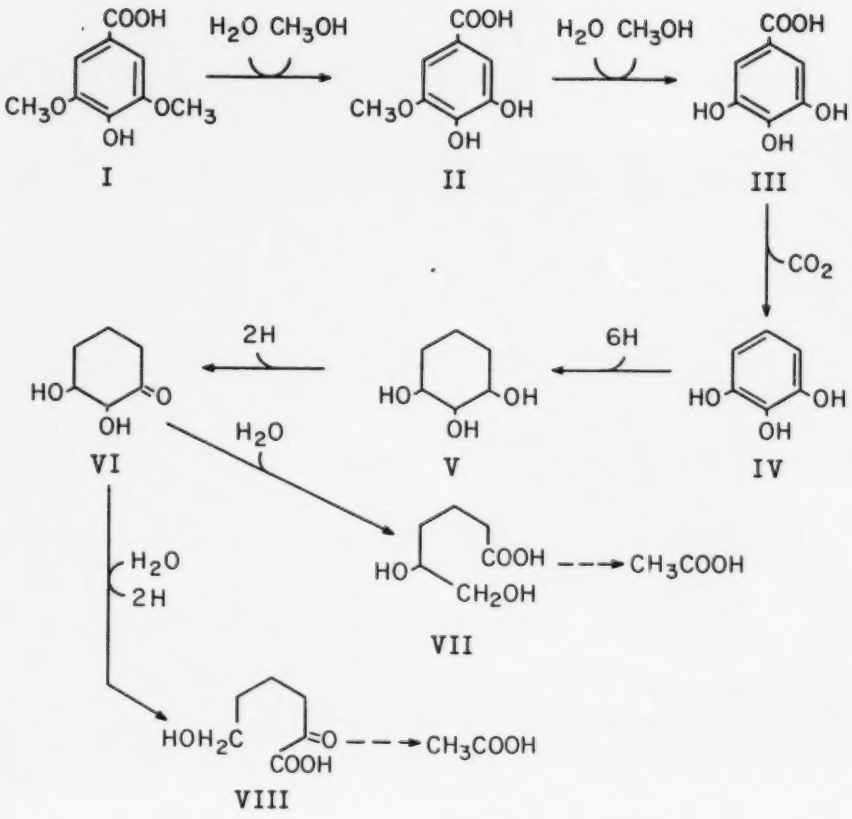

Fig. 6. Proposed pathway for the degradation of syringic acid by a methanogenic consortium. Compounds are: (I) syringic acid, (II) 5-methoxy-3,4-dihydroxybenzoic acid, (III) gallic acid, (IV) pyrogallol, (VI) 1,2,3-trihydroxycyclohexane, (VI) 2,3-dihydroxcyclohexanone, (VII) 5,6-dihydroxyhexanoic acid, (VIII) 6-hydroxy-2-oxohexanoic acid.

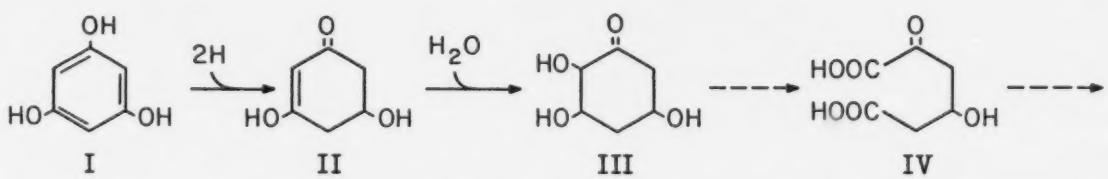

Fig. 7. Proposed pathway for the degradation of phloroglucinol. Compounds are: (I) phloroglucinol, (II) dihydrophloroglucinol, (III) 2,3,5-trihydroxycyclcohexanone, (IV) 2-oxo-4-hydroxyadipic acid.

degradation step. The anaerobic degradation of various chlorophenols was studied in groundwater aquifer microcosms (Suflita and Miller 1985). Microorganisms in actively methanogenic aquifer material initiated the degradation of chlorinated phenols by reductive dechlorination. Complete removal of the chlorine moieties occurred before the phenol intermediate could be mineralized to methane and carbon dioxide. Methanogenic activities were apparently required for the loss of chlorine because the chlorinated phenols persisted in anoxic microcosms containing material from the same aquifer but from a nonmethanogenic site. The reductive dehalogenation of chloro-, bromo- and iodo-benzoates was demonstrated in methane-producing fresh water lake sediment (Horowitz et al. 1983). Dehalogenation occurred after a lag period which lasted from 1 week to more than 6 months, depending on the chemical.

Horowitz et al. (1983) have suggested that acclimation of sediments to dehalogenation and mineralization of the aromatic ring may be independent processes. They reached this conclusion from three lines of evidence. Firstly, acclimation was observed regardless of whether the parent substrate was eventually mineralized to carbon dioxide and methane. Secondly, the pattern of dehalogenation of consortia acclimated to different halogenated benzoates was unaffected by prior acclimation of the sediment to benzoate. Lastly, acclimation to dehalogenation was not concurrent with acclimation to benzoate mineralization. This last piece of evidence also tends to support a degradative mechanism (Fig 8). where dehalogenation is required before a substrate can be degraded to carbon dioxide and methane.

Such a pathway may not be restricted to a single class of compounds. Boyd and Shelton (1984) observed the sequential dehalogenation of several dichlorinated phenols, and an anaerobic consortium was shown to convert (2,4,5-trichlorophenoxy)acetic acid to (2,5-dichlorophenoxy) acetic acid (Suflita et al. 1984) In much earlier studies, pentachlorophenol was shown to be degraded (Ide et al. 1972, Murthy et al. 1979) in anaerobic soil to isomeric mixtures of partially dehalogenated phenolic intermediates, and Kirkpatrick et al. (1981) showed that the bactericide N-(2,3-dichlorophenyl)-3,4,5,6,-tetrachlorophthalamic acid was metabolized to two and possibly more monodechlorinated products. Apparently for halogenated benzoates and other compounds, dehalogenation and mineralization of the benzoate ring are, likely, independent processes and dehalogenation occurs before degradation of the rest of the compound.

Not all chlorinated compounds are dechlorinated as the initial step in anaerobic degradation. Chloroguaiacols and chloroveratroles have recently been reported (Remberger et al. 1986) to be de-O-methylated to the corresponding chlorocatechols. The fate of the chlorocatecols was not resolved.

Halogenated aliphatic compounds

The initial step in the degradation of halogenated aliphatic compounds is reductive dehalogenation. Unlike halogenated aromatic compounds, however, halogenated aliphatics can lose one or two halides during the reductive process. The mechanism for the loss of two halogens involves the removal of two vicinal halogens with the formation of a double bond (Kray and Castro 1964, Beland et al. 1976). The reductive loss of a single halide is the same for both aromatic

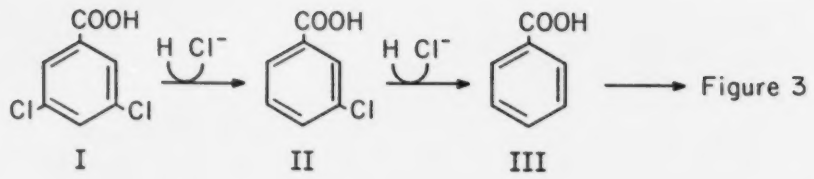

Fig. 8. Proposed pathway for the degradation of 3,5-dichlorobenzoic acid by a methanogenic consortium. Compounds are: (I) 3,5-dichlorobenzoic acid, (II) 3-chlorobenzoic acid, (III) benzoic acid.

and aliphatic compounds, involving the replacement of the halogen with a hydrogen atom (Essac and Matsumura 1980).

Vicinal dehalogenation of several potentially hazardous compounds has been reported. Castro and Belser (1968) found that flooded soil cultures stoichiometrically converted ethylene dibromide (1,2-dibromoethane) to ethylene. They also were able to demonstrate the conversion of 2,3-dibromobutane to 2,3-butene. Examination of the products of the debromination of the d,l- and meso-isomers indicated trans elimination of the two bromide ions. The vicinal dechlorination of lindane to 3,4,5,6-tetrachlorocyclohexene has been demonstrated in sewage sludge (Beland et al. 1976), in pure cultures of anaerobic and facultative anaerobic bacteria (MacRae et al. 1969, Tu 1976, Heritage and MacRae 1977a, Jagnow et al. 1977) and in cell free preparations of Clostridium sphenoides (Heritage and Mac Rae 1977b). Beland et al. (1976) suggested that vicinal dechlorination was the first step in the degradation of lindane in sewage sludge and that this reaction was followed by complete dechlorination to yield benzene. Benzene was observed at trace levels in sewage sludge amended with lindane but not in unamended cultures. The tetrachlorinated intermediate and benzene also were observed in electrochemical reduction experiments. This observation led Beland et al. (1979) to suggest that electrochemical data could be used to predict anaerobic degradation products of organohalogen compounds in the environment. The anaerobic degradation of lindane in flooded soils is apparently more complicated and may proceed by discrete steps involving the loss of a single halogen with subsequent aromatization of the ring. Mathur and Saha (1975) isolated and identified by gas chromatography and mass spectroscopy the degradation products 3,4,5,6-tetrachlorocyclohexane (>5% of added lindane), 2,3,4,5,6-pentachlorocyclohexane (2%) and trace amounts of 1,2,4-trichlorobenzene and 1,2,3,5- and/or 1,2,4,5-tetrachlorobenzene.

Reductive dehalogenation by replacement of a single halogen with a hydrogen atom also has a prominent role in the degradation of halogenated aliphatic compounds. For example, DDT [1,1,1-trichloro-2,2-bis (parachlorophenol) ethane] is converted to DDD [1,1-dichloro-2,2-bis (parachlorophenol) ethane] in flooded soil or in moist soil incubated anaerobically (Spencer 1967, Hill and McCarty 1967, Guenzi and Beard 1968, Ko and Lockwood 1968, Kearney et al. 1969, Castro and Yashida 1971). Chloroform has been observed as an intermediate in the degradation of carbon tetrachloride under nitrifying conditions (Bouwer and McCarty 1983) and in anaerobic subsurface aquifer microcosms (Parsons et al. 1985, Parsons and Lage 1985). Parsons and Lage (1985) also reported that 1,1,1-trichloroethane was converted to 1,1-dichloroethane. Data reported from a number of laboratories indicates that tetrachloroethylene is sequentially dechlorinated under anaerobic conditions by a series of one chlorine extractions to yield vinyl chloride, which is then degraded to CO_2 (Fig. 9). Bouwer and McCarty (1983) showed that, in methanogenic bacterial cultures, the initial step in the transformations of tetrachloroethylene and 1,1,2,2-tetrachloroethane to nonchlorinated end products appeared to be reductive dechlorination to trichloroethylene and 1,1,2-trichloroethane. In later studies, Parsons et al. (1984) reported that depletion of tetrachloroethylene and appearance of cis- and trans-1,2-dichloroethylene and vinyl chloride were observed following incubation of tetrachloroethylene in microcosms containing material from an aquifer recharge basin. Kleopfer et al. (1985) observed the reductive dechlorination of

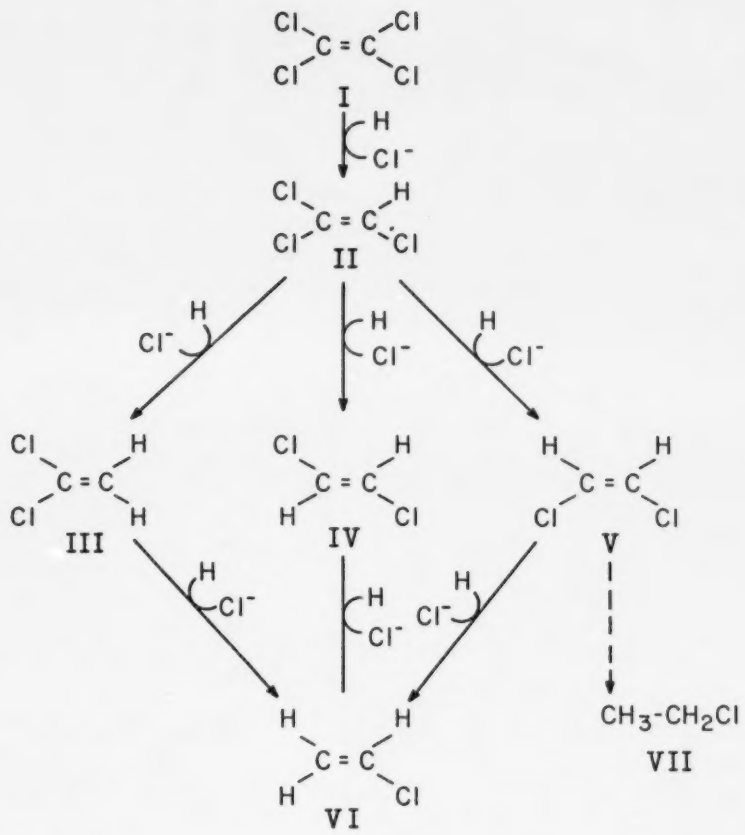

Fig. 9. Proposed pathway for the degradation of tetrachloroethylene by anaerobic bacterial consortia. Compounds are: (I) tetrachloroethylene, (II) trichloroethylene, (III) 1,1-dichloroethylene, (IV) trans-dichloroethylene, (V) cis-dichloroethylene, (VI) vinyl-chloride, (VII) chloroethane.

trichloroethylene to 1,2-dichloroethylene in anaerobic soil samples. No 1,1-dichloroethylene was observed. The degradation of cis- and trans-dichloroethylene was examined in anaerobic subsurface aquifer materials (Barrio-Lage et al. 1986). Vinyl chloride was produced in all amended microcosms and was not observed in sterile or unamended controls. Chloroethane was produced only in microcosms amended with the cis isomer. Vogel and McCarty (1985) found that 24% of the tetrachloroethylene was mineralized in a continuous-flow fixed-film methanogenic column study. Trichloroethylene was the major intermediate, but traces of dichloroethylene isomers and vinyl chloride also were found. Under a different set of methanogenic conditions, nearly quantitative conversion of tetrachloroethylene to vinyl chloride was observed.

Sufficient evidence exists to suggest that some anaerobic dehalogenations are not strictly biological, but are catalyzed by free iron, other metal ions or iron porphyrins in reducing environments. Glass (1972) postulated that reduction of DDT to DDD in soils is mediated by the iron (Fe+2/Fe+3) couple. The reduction of DDT in an aqueous system containing hematin and sodium dithionate has been demonstrated by Miskus et al. (1965), and Zoro et al. (1974) has extended this work to the degradation of DDT by iron porphyrins. Zoro et al. (1974) also observed that, when dithionate was added to sterilized sewage sludge or yeast suspensions, dehalogenation activity was either restored or enhanced. These authors have suggested that under anaerobic conditions in the environment, cell-free reduced iron porphyrins (released from decaying organisms) are responsible for the reductive dechlorination of DDT. Presumably, the active organisms participated in the degradation process by maintaining a strongly reducing environment and providing a source of iron porphyrins.

Nonspecific reactions are not limited to DDT. Subsequent studies by Holmstead (1976) and Khalifa et al. (1976) have demonstrated the reductive dehalogenation of toxaphene and mirex by iron porphyrins. More recently, Klecka and Gonsior (1984) observed that iron (II) porphyrins reduced carbon tetrachloride, chloroform, and 1,1,1-trichloroethane to lower chlorinated homologs. Solutions of chromium salts have been reported (Kray and Castro 1964) to catalize dehalogenation of vicinal halides to olefins. The membrane fraction of cell-free extracts of Clostridium sphenoides catalyzed the vicinal dechlorination of lindane to 3,4,5,6-tetrachloro-1-cyclohexene if reduced glutathione was added to the reaction mixture (Heritage and MacRae 1977b). Bouwer et al. (1981) found that brominated trihalomethanes were degraded rapidly in both sterilized and active mixed methanogenic cultures.

Aromatic and aliphatic hydrocarbons

Although a limited number of reports describing the degradation of hydrocarbons have appeared in the literature, these pollutants are generally believed to be resistant to degradation in the absence of oxygen. Unfortunately many of the early reports suffered from inadequate anaerobic techniques, poorly defined culture conditions, mixed cultures of aerobic and anaerobic bacteria, and substrates potentially contaminated with readily degradable compounds (Novelli and Zobell 1944, Rosenfeld 1947, Muller 1957, Davis and Yarbrough 1966). Within the last year, however, two definitive studies have

appeared describing the degradation of both aliphatic (Schink 1985) and aromatic (Grbic-Galic and Vogel 1986) hydrocarbons.

Schink (1985) investigated the degradation of hydrocarbons in anaerobic enrichments using mineral media inoculated with sewage sludge or sediment samples of limnic or marine origin. Complete degradation of 1-hexadecene was observed; squalene was only partially degraded to carbon dioxide and methane. Methanogenic enrichments were maintained on either squalene or 1-hexadecene. No indication of methanogenic degradation was obtained with either n-hexane, n-hexadecane, n-heptadecane, 1-hexene, cis-2-hexene, trans-2-hexene, isoprene, 1-hexine, benzene, toluene, xylene, cyclohexene, cycloheptatriene, cyclopentadiene, styrene, naphthalene, azulene, or β-carotene. Schink concluded that, under his assay conditions, a terminal double bond can be sufficient to allow methanogenic degradation of hydrocarbons, whereas branching and terminal ring closures may significantly contribute to hydrocarbon stability in anoxic sediments. Although no degradation of linear saturated hydrocarbons was observed in this study, Giger et al. (1980) observed the degradation of heptadecane in anoxic lake sediment samples and Hambrick III et al. (1980) observed the degradation of octadecane in anaerobic sediment-water suspensions.

Only recently has there been an indication that aromatic hydrocarbons can be degraded under anaerobic conditions. Two reports (Batterman and Werner 1984, Kuhn et al. 1985) have appeared decribing the degradation of aromatic compounds under denitrifying conditions. Batterman and Werner (1984) were able to effect anaerobic degradation of toluene, benzene, and xylenes in a contaminated aquifer by injecting nitrate. Kuhn et al. (1985) observed anaerobic degradation of xylenes under denitrifying conditions in the field and in laboratory aquifer columns simulating a river water/groundwater infiltration system. The p- and m-xylene isomers were degraded at similar rates and significantly faster than the o-isomer. In an extensive laboratory study using methanogenic cultures acclimated to the degradation of ferulic acid, Grbic-Galic and Vogel (1986) demonstrated that benzene, toluene and o-xylene were first oxidized to phenolic and carboxylic acid intermediates before the compounds could be degraded by standard ring reduction pathways (Evans 1977). Using ^{18}O labelled water, Vogel and Grbic-Galic (1986) demonstrated that oxygen incorporated into the ring came from water and not methanol (used to add substrates) or molecular oxygen that may have contaminated the sample.

Nitrogen containing compounds

Nitrogen containing organic compounds can be anaerobically transformed by a number of reaction mechanisms that include nitro reduction, N-dealkylation, azo reduction and the reduction of nitrogen heterocycles with subsequent ring cleavage.

The primary route for anaerobic degradation of nitro substituted xenobiotic compounds is reduction of the nitro-group to the corresponding amino substituent. Soleim and Schelini (1972) reported that hydroxyamino substituents may be intermediates in the reduction process. They have identified p-hydoxyaminobenzoate and p-aminobenzoate in cultures of anaerobic strains of Clostridia, Bacteriodes, and other anaerobes metabolizing p-nitrobenzoate. McCormick et al. (1976)

observed that the reduction of several nitro aromatic compounds followed the mechanism proposed by Yamashina et al. (1954) for the enzymatic reduction of aromatic nitro, nitroso, and hydroxylamine compounds (Fig. 10). For compounds that have multiple nitro-substituents, sequential reduction of the nitro groups occurs. For example, for trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N dipropyl-p-toluidine) reduction of one of the nitro groups is followed by reduction of the other nitro group and/or dealkylation of one or both propyl groups (Probst et al. 1967, Golab et al. 1969, Williams and Fiel 1971, Parr and Smith 1973). Benifin (N-butyl-N-ethyl-a,a,a-trifluoro-2,6-dinitro-p-toluidine) is also degraded in a similar manner (Golab et al. 1970). The nitro groups are sequentially reduced to amino groups and one or both of the alkyl groups may be lost. Substituent specificity also was demonstrated for 2,4,6-trinitrotoluene and 2,4-dinitrotoluene where the 4-nitro group is reduced before the 2-or 6 nitro groups (McCormick et al. 1976). Little is known of the fate of the amino intermediates in the degradation of these compounds. In many cases they appear to be persistent (Williams 1977); however, Braun and Gibson (1984) and Shelton and Tiedje (1984a) have demonstrated that aminobenzoates can be mineralized under denitrifying and methanogenic conditions.

$$R\text{-}NO_2 \xrightarrow{H_2} R\text{-}NO \xrightarrow{H_2} R\text{-}NHOH \xrightarrow{H_2} R\text{-}NH_2$$

Figure 10. Mechanism for the reduction of nitro compounds.

N-dealkylation by anaerobic ruminal and flooded soil microorganisms has been reported for trifluralin (Williams and Feil 1971, Parr and Smith 1973) and for benefin incubated in ruminal solutions (Golab et al. 1970). Several of the trifluralin and benefin metabolites were characterized as compounds lacking one or both of the N-alkyl groups, and it was postulated that N-dealkylation was a result of anaerobic microorganisms.

The initial reaction (Fig. 11) in the degradation of azo compounds by anaerobic microorganisms is the four electron reduction of the azo linkage to yield two primary amines (Wuhrmann et al. 1980).

$$(R_1\text{-}N{=}N\text{-}R_2) + 4e^- + 4H^+ \longrightarrow R_1\text{-}NH_2 + R_2\text{-}NH_2$$

Figure 11. Mechanism for the reduction of azo compounds.

Although previous studies had indicated that azo compound reduction occurred on the outside of anaerobic microorganisms (Dubin and Wright 1975), more recent results indicate that reduction is an activity internal to the microorganisms (Wuhrmann et al. 1980). Wuhrmann et al. (1980) found that a series of azo dyes having different permeability properties were degraded at similar rates by cell extracts and were degraded at markedly dissimilar rates by whole cells. These authors proposed that their results were consistent with the mechanism advanced

by Gingell and Walker (1971) which proposed an intracellular, nonenzymatic reduction of azo compounds by reduced flavin nucleotides.

The number of nitrogen heterocycles known to be degraded anaerobically include indole (Wang et al. 1984), nicotinic acid (Harary 1957, Stadtman et al. 1972, Imhoff and Andressen 1979, Imhoff-Stuckle and Pfennig 1983), and alkyl pyridines (Rogers et al. 1985). Wang et al. (1984) demonstrated that indole was anaerobically degraded to methane, carbon dioxide, and ammonium ion. Based on the amount of indole added and the stoichiometric relationship in Fig. 12,

$$C_8H_7N + 8H_2O \longrightarrow 3.5CO_2 + 4.5CH_4 + NH_4^+ + OH^-$$

Figure 12. Stoichiometry of anaerobic indole degradation.

they were able to recover 84.4% of the methane that would be produced theoretically. Harary (1957) was the first to demonstrate anaerobic degradation of a nitrogen heterocycle. He showed that nicotinic acid was fermented to acetate, propionate, ammonia, and carbon dioxide. Subsequently, Tsai et al. (1966) isolated and charaterized the metabolic intermediates shown in Fig. 13. The aerobic and anaerobic degradation of a mixture of alkyl pyridines in a groundwater sample were investigated by Rogers et al. (1985). A marked difference was observed between the aerobic and anaerobic degradation rates. Under aerobic conditions, the residual alkylpyridine concentrations generally approached zero concentration within 10 to 31 days; whereas under anaerobic conditions the concentrations of residual alkylpyridines decreased between 40 and 80% over 33 days. Biodegradation rates under aerobic conditions were greatly affected by the specific ring substitution of structural isomers. A similar effect was not observed for anaerobic degradation rates.

Ether linked compounds

Methanogenic enrichments capable of degrading a series of polyethylene glycols and ethylene glycol have been isolated from sewage sludge (Dwyer and Tiedje 1983). The enrichments were shown to best degrade glycols close to the molecular weight on which they were enriched. Ethanol, acetate, methane, and ethylene glycol were detected as products. The sequence of product formation suggested that the ethylene oxide unit $[HO-(CH_2-CH_2-O-)xH$ was dismutated to acetate and ethanol; ethanol was subsequently oxidized to acetate by a syntrophic association that produced methane.

ENVIRONMENTAL INTERACTIONS

Although the degradation of many hazardous organic chemicals has been conclusively established in laboratory studies, extrapolation of this work to the environment is only beginning to be explored. There is a general lack of information on the concentration and turnover rate of hazardous organic chemicals in anaerobic environments, and, unfortunately, the effect of environmental parameters on degradative processes also has received little attention.

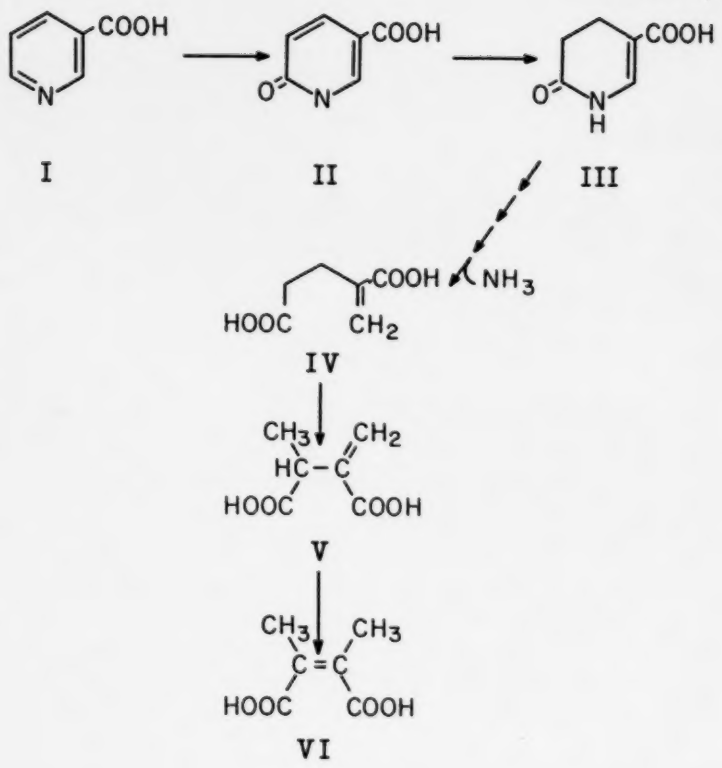

Fig. 13. Proposed pathway for the degradation of nicotinic acid by Clostridium barkeri sp. n. Compounds are: (I) nicotinic acid, (II) 6-oxonicotinic acid, (III) 1,4,5,6-tetrahydro-6-oxonicotinic acid, (IV) 2-methylene-glutaric acid, (VI) methylitaconic acid, (VI) dimethylmaleic acid.

The limited information available is scattered. For example, Rajaram and Sethunathan (1975) observed that organic carbon sources accelerated nitro group reduction of parathion in flooded soils. Degradation rates followed the order glucose > rice straw > algal crust > farmyard manure > unamended. Capone et al. (1983) examined the effects of several metals on microbial methane, carbon dioxide and sulfide production in sediments from Spartina alternifora communities. Comparisons were made between control and experimental assays with respect to initial rates of production (after acclimation) and overall production. Methane evolution was inhibited by CH_3HgCl, HgS, and $NaAsO_2$. A period of initial inhibition was followed by a period of overall stimulation with the chlorides of Hg, Pb, Ni, Cd and Cu, and with $ZnSO_4$, K_2CrO_4, and $K_2Cr_2O_7$. Production of CO_2 was generally less affected by the addition of metals. Inhibition was noted with $NaAsO_2$, CH_3HgCl, and Na_2MoO_4, and minor stimulation of CO_2 production occurred over the long term with chlorides of Hg, Pb, and Fe. Sulfate reduction was inhibited in the short term by all metals tested and over the long term by all but $FeCl_2$ and $NiCl_2$. Several studies have indicated that in aerobic sediments that both oxygen- and nitrate-dependent aromatic metabolism can occur. Fabig et al. (1980) found that, in the presence of trace amounts of oxygen, benzoate degradation and nitrate reduction occurred concurrently in either a Pseudomonas aeruginosa or an undefined mixed culture; they were unable to demonstrate nitrate-dependent benzoate degradation under strictly anaerobic conditions. Sleat (1981) demonstrated that the growth of nitrate-reducing facultative anaerobes was increased by the presence of nitrate when they were grown on p-hydroxybenzoate under trace oxygen concentrations. Jones (1979) has suggested that microzones within particles were responsible for nitrate reductase activity in aerobic freshwater sediments, and Focht and Verstraete (1977) considered such anoxic microzones as the sites of denitrification in aerobic sediments.

In examining the anaerobic degradation of benzoate in undiluted sediments, Sleat and Robinson (1983) observed that the addition 10 mM sulfate did not inhibit the rate of ^{14}C-labelled gas production but did alter the ratio of methane to carbon dioxide. In the presence of sulfate, only carbon dioxide was produced and in its absence a mixture of methane and carbon dioxide indicative of methanogenic activity was observed. These results are consistent with results of several other investigators (Cappenberg 1974, MacGregor and Keeney 1973, Winfrey and Zeikus 1977) who have shown that sulfate inhibits methanogenesis in freshwater sediments. Greater affinities for H_2 or acetate (Abram and Nedwell 1978a,b; Bryant et al. 1977; McInerney et al. 1979; Ward and Olson 1980; Winfrey and Zeikus 1977), the minimum threshold for hydrogen metabolism in methanogenic bacteria (Lovley et al. 1982; Lovley 1985) and bioenergetic reasons (Zehnder 1978; Lovley et al. 1982) have been invoked to explain the competitive advantage of sulfidogens over methanogens. Whatever the true mechanism, sulfate-reducing bacteria appear to assume the role of methanogenic bacteria in sulfate-containing sediments by using methanogenic precursors. The fact that gas production from benzoate was not affected by sulfate in Sleat and Robinson's work would suggest that ring cleavage or the production of methanogenic precursors was the rate limiting step in the presence and absence of sulfate.

METHODS OF INVESTIGATION

Current methods used to investigate the anaerobic degradation and transformation of potentially hazardous chemicals in the environment range

from the classical Hungate (1969) techniques, using pure bacterial cultures or suspensions of sewage sludge, sediment, or aquifer materials, to more modern techniques that attempt to use sediment and aquifer materials in an undisturbed state.

Classical anaerobic techniques have been the workhorse of countless scientific investigations since the original decription of the "Hungate" technique in 1950 (Hungate 1950). Since that time the technique has undergone numerous modifications and improvements (Bryant 1972, Miller and Wolin 1974, Balch and Wolfe 1976). This method uses glass anaerobic culture tubes, serum bottles or various glass containers fitted with serum bottle necks that are tightly sealed with rubber stoppers and a crimped metal seal. Neoprene, butyl, or synthetic, but not gum, rubber stoppers are suitable. If an anaerobic chamber is available the addition of anaerobic sewage sludge, sediment or aquifer materials, hazardous chemicals and other necessary solutions are added in the chamber and the glass container is sealed before removal. If a chamber is not available, additions are made to glass containers that are continually gassed with O_2 free gases. The stoppers are inserted as the bottles are withdrawn from the gassing needles.

Anaerobic techniques of this nature combined with analytical organic procedures have been used to investigate the fate of hazardous organic chemicals in the environment. Most studies have followed classical microbiological approaches and have used pure cultures or acclimated or fresh sediment, sewage sludge, or aquifer material in suspension to investigate the mechanisms of anaerobic transformations and anaerobic degradative pathways (e.g. Bakker 1977, Boyd and Shelton 1984, Healy and Young 1979), to identify the microorganisms active in the degradative process (Huser et al. 1982, Frazer and Young 1985, Harwood and Gibson 1986), and to provide evidence for kinetic mechanisms mathematically describing the persistance of these compounds (Suflita et al. 1983).

Shelton and Tiedje (1984a), for example, have proposed a simple, general method to test whether an organic chemical was susceptible to anaerobic degradation to CH_4 and CO_2. The method uses digested sewage sludge diluted to 10% and incubated anaerobically in 160-ml serum bottles with 50 µg of C per ml of test chemical. Biodegradation was determined after 8 weeks by the net increase in gas pressure in bottles with test chemicals over the pressure in nonamended sludge bottles. Gas pressure could be measured by a pressure transducer. Alternatively the increase in CH_4 or CO_2 could be measured in amended sample by GC. Shelton and Tiedje cautioned that this general screening method only measures degradation potential. In a particular anaerobic environment, the potential may not be expressed if degrading organisms are absent or if toxic chemicals or other environmental factors limit the organisms activity. Degradation potential should not be construed as the actual rate that a compound is degraded if released to the environment.

For many studies a long lag period, where a loss in compound cannot be detected, precedes degradation of the compound. This time interval has been called an "adaptation" period (Clark and Fina 1952), a "time lag" (Balba and Evans 1977) and an "acclimation lag" (Healy and Young 1979). Sleat and Robinson

Robinson (1984) have suggested that research into the anaerobic degradation of ardous organic chemicals has been retarded by the belief that anaerobic degradation, particularly the methanogenic fermentation, requires a long lag period before detectable loss of the study compound is observed.

The importance of the acclimation period may be overstated. In earlier studies, Sleat and Robinson (1983) showed that the length of the acclimation periods are the result of sample preparation. Increasing the sediment to water ratio markedly reduced the acclimation period. When benzoate was added to a 10% suspension of sediment, methanogenesis was observed after acclimation periods ranging from 50 to 80 days. In undiluted sediments amended with [ring-U-C14] benzoate, ^{14}C-labelled gas production was detected after as little as 4 hours of anaerobic incubation. They also found that the temperature optimum for benzoate mineralization dropped from 37°C to 28°C when undiluted sediments were used instead of diluted sediments. The difference in temperature optima apparently resulted from the a change in the rate limiting step in the conversion of benzoate to methane and carbon dioxide. In undiluted samples ring cleavage or the formation of methanogenic precursors is the rate limiting process whereas in diluted samples methanogenesis is the rate limiting step. Sleat and Robinson (1984) suggested that undiluted samples may be more appropriate for examining environmental effects on anaerobic degradation processes.

Other investigators have also advocated the use of undiluted sediment or undisturbed sediment cores in examining the anaerobic degradation or transformation of organic and inorganic compounds. Jorgensen (1978) found that disturbing sediment cores by mixing resulted in a small loss of activity whereas dilution of the sediment caused a marked decrease in the rate of sulfate reduction calculated per unit volume of undiluted sediment. Undiluted mixed sediment had two-fold lower activity sediment cores. A 1:10 dilution of sediment resulted in a 25-fold decrease in activity when compared to sediment cores. In a second study Jorgenson (1978) found no difference in sulfate reduction for sediments that were directly injected with labelled sulfate; that were pooled in a vessel, carefully mixed, cored again, and then injected; or that were pooled, carefully mixed with labelled sulfate, and cored again. When repeated at 14°C, the mixed incubations were somewhat lower than the injected samples, although the difference was significantly less than the two-fold difference found in the initial experiment. Jones and Simon (1984) observed similar results when they examined the mineralization of acetate in small sediment cores. They cautioned, however that the effects of sorption and diffusion within the sediment cores should be closely examined.

CONCLUSIONS

The anaerobic degradation of potentially hazardous chemicals released to the environment has received increased attention over the last 10 to 20 years. This increased interest has led to significant advances in identifying the principal microorganisms involved in the anaerobic degradation process, unravelling the complex degradative pathways used by these organisms, and demonstrating the potential for anaerobic degradation of a large number of compounds. As these studies have developed, a number of key processes have been recognized. Several common degradative pathways appear to be shared by

all bacteria and bacterial communities capable of anaerobic degradative activity. Ring reduction precedes ring cleavage in the degradation of aromatic compounds. Substitutents of aromatic compounds are generally removed to produce benzoate or phenol before ring reduction and ring cleavage occurs. In most substrates, including sewage sludge, sediments, and aquifer material, degradation results from the activity of microbial consortia having methanogenic and sulfidogenic organisms acting as the final electron acceptors of the community. The removal of ring substituents, ring reduction and ring cleavage is generally performed by fermentative members of the consortia.

Despite these advances there is a lack of information on the turnover rates of hazardous organic chemicals in anaerobic environments. The lack of attention apparently results from the difficulties in working with bacteria and bacterial communities under strict anaerobic conditions, the long adaptation periods before degradation is observed for many compounds and the statistical problems inherent in working with undisturbed sediments (Jones and Simon 1984).

Work in this area may, however, be facilitated by the increasing number of aerobic studies directed toward prediction of the persistence and concentration of toxic chemicals in natural environments. A number of kinetic models have been proposed to characterize degradation in natural environments (Paris et al. 1981, Vashon and Schwab 1982, Lewis et al. 1984) and in pure cultures of microorganisms (Simkins and Alexander 1984, Robinson 1985). Many of these models are based on the assumption that substrate disappearance can be modeled with information only on substrate concentration and population density together with parameters of classical Monod kinetics (Simkins and Alexander 1984). Recently, models were described for the kinetics of biodegradation of organic compounds of bacteria growing on an alternative carbon source (Schmidt et al. 1985). Statistical means have been proposed for evaluating the fits of these models to data obtained from laboratory studies.

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Table 1. Comprehensive List of Compounds Microbially Degraded or Transformed under Anaerobic Conditions.

Compound	Reference	Microorganism(s)	Degradation/Transformation
acetyl salicyclic acid	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
acrylic acid	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
alkyl pyridines	Rogers et al., 85	subsurface waters & soils	transformation[a]
2-aminobenzoate	Shelton & Tiedje, 84a Braun & Gibson, 84	dilute sewage sludge *Pseudomonas* sp.	deg. to CH_4 & CO_2 mineralization[b]
4-aminobenzoate	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
4-amino-3-chlorobenzoate	Suflita et al., 82	dilute sewage sludge dilute sediment	dechlorination
4-amino-3-5-dichlorobenzoic acid	Suflita et al., 83	dilute sediment 3-chlorobenzoate enrich. meth. cult.	dechlorination
	Suflita et al., 82	dilute sewage sludge dilute sediment	dechlorination
amoranth	Dubin & Wright, 75	*Proteus vulgaris*	reduction
m-anisic acid	Taylor, 83 Shelton & Tiedje, 84a	*Pseudomonas* sp. Strain PN-1 dilute sewage sludge	demethylation deg. to CH_4 & CO_2

[a] In the context of this table transformation indicates that the investigators monitored the disappearance of the compound and no indication was given to indicate partial or complete degradation of the compound.

[b] Mineralization is used to indicate that the investigators followed formation of CO_2. The compound was completely degraded with a constant fraction converted to CO_2 and the remainder converted to biomass.

Compound	Reference	Microorganism(s)	Degradation/Transformation
anisol	Bache & Pfennig, 81	*Acetobacterium woodii*	demethylation
benefin	Golab et al., 70	flooded soil	transformation
benzidine-based azo dyes	Manning et al., 85	dilute human feces	azo reduction
benzene	Grbic-Galic & Vogel, 86	ferulic acid enriched methanogenic consortium	deg. to CH_4 & CO_2
benzo(*a*)pyrene	Delaune et al., 81	dilute sediment	deg. to CH_4 & CO_2
benzoate	Harwood & Gibson, 86	*Rhodopseudomonas palustris*	mineralization
	Fina & Fiskin, 60	bovine rumen fluid	
		dilute sewage sludge	deg. to CH_4 & CO_2
	Proctor & Scher, 60	*Rhodopseudomonas* sp	mineralization
	Guyer & Hegeman, 69	*Rhodopseudomonas palustris*	mineralization
	Dutton & Evans, 69	*Rhodopseudomonas palustris*	mineralization
	Dutton & Evans, 69	*Rhodopseudomonas palustris*	transformation
	Nottingham & Hungate, 69	enriched methanogenic cultures	deg. to CH_4 & CO_2
	Williams & Evans, 75	*Moraxella* sp.	mineralization
	Ferry & Wolfe, 76	benzoate enriched methanogenic culture	deg. to CH_4 & CO_2
	Balba & Evans, 77	enriched methanogenic culture	deg. to CH_4 & CO_2
	Evans, 77	*Rhodopseudomonas palustris*	deg. to CH_4 & CO_2
		Pseudomonas strain (NPI) enriched methanogenic cultures	
	Bakker, 77	enriched nitrifying cultures	transformation
	Fina et al., 78	enriched methanogenic culture	deg. to CH_4 & CO_2
	Keith et al., 78	benzoate enriched methanogenic cultures	transformation
	Shlomei et al., 78	benzoate enriched methanogenic cultures	transformation
	Healy & Young, 79	enriched meth. cultures	deg. to CH_4 and CO_2
	Balba & Evans, 80	mixed cultures: *P. aeruginosa* & *D. vulgaris*	
	Mountfort & Bryant, 82	cocultures of an anaerobic bacterium with H_2 utilizing organism	deg. to CH_4 & CO_2

Compound	Reference	Microorganism(s)	Degradation/Transformation
benzoate (cont)	Sleat & Robinson, 84	freshwater sediment	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
	Harwood & Gibson, 86	Rhodopseudomonas palustris	mineralization
	Tarvin & Buswell, 34	dilute sewage sludge	deg. to CH_4 & CO_2
benzyl alcohol	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
brominated trihalomethanes	Bouwer et al., 81	dilute sewage sludge	debromination
	Bouwer & McCarty, 83b	enriched nitrifying culture	transformation
bromodichloro methane	Bouwer & McCarty, 83b	enriched nitrifying culture	transformation
bromoform	Bouwer & McCarty, 83b	enriched nitrifying culture	transformation
2-bromobenzoate	Suflita et al., 82	dilute sewage sludge dilute sediment	deg. to CH_4 & CO_2
3-bromobenzoate	Horowitz et al., 83	dilute freshwater sediments	debromination deg. to CH_4 & CO_2
	Suflita et al., 82	dilute sewage sludge dilute sediment	deg. to CH_4 & CO_2
4-bromobenzoate	Suflita et al., 82	dilute sewage sludge dilute sediment	deg. to CH_4 & CO_2
2,3-butanediol	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
butylbenzyl phthalate	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
caffeic acid	Perez-Silva et al., 66	rat feces	dehydroxylation
carbon tetrachloride	Bouwer & McCarty, 83a	enriched methanogenic culture	transformation
	Bouwer & McCarty, 83b	enriched nitrifying culture	transformation

Compound	Reference	Microorganism(s)	Degradation/Transformation
catechol	Healy & Young, 78	dilute sewage sludge	deg. to CH_4 & CO_2
4-chloroacetanilide	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
3-chlorobenzoic acid	Suflita et al., 83	dilute sediment 3-chlorobenzoate enriched methanogenic community	dechlorination
	Suflita et al., 82	dilute sewage sludge dilute sediment	dechlorination
	Shelton & Tiedje, 84b	dilute sewage sludge	deg. to CH_4 & CO_2
Chloroethene	Parsons et al., 85	groundwater materials	transformation
Chloroform	Bouwer & McCarty, 83b	enriched nitrifying culture	transformation
2-chlorophenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84	dilute sewage sludge	deg. to CH_4 & CO_2
	Suflita & Miller, 85	aquifer materials	deg. to CH_4 & CO_2
3-chlorophenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Suflita et al., 82	dilute sewage sludge dilute sediment	deg. to CH_4 & CO_2
	Suflita & Miller, 85	aquifer materials	deg. to CH_4 & CO_2
4-chlorophenol	Suflita & Miller, 85	groundwater materials	deg. to CH_4 & CO_2
4-chlorophthalic acid	Taylor & Ribbons, 83	a marine mixed culture	decarboxylation
cholesterol	Taylor et al., 81	*Pseudomonas* sp. strain PN-1	demethylation
cinnamic acid	Healy & Young, 79	dilute sewage sludge	deg. to CH_4 & CO_2
	Tarvin & Buswell, 34	dilute sewage sludge	deg. to CH_4 & CO_2
coniferyl alcohol	Grbic-Galic, 83	ferulic acid enriched enriched methanogenic culture	deg. to CH_4 & CO_2

Compound	Reference	Microorganism(s)	Degradation/Transformation
m-cresol	Bakker, 77	enriched nitrifying culture	transformation
	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Fedorak & Hrudey, 84	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
o-cresol	Bakker, 77	enriched nitrifying culture	transformation
	Fedorak & Hrudey, 84	dilute sewage sludge	deg. to CH_4 & CO_2
p-cresol	Bakker, 77	enriched nitrifying culture	transformation
	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
DDT	Miskus et al., 65	rumen fluid	dechlorination
	Glass, 72	flooded soil	dechlorination
	Sethunathan, 73	flooded soil	transformation
	Zoro et al., 74	dilute sewage sludge	dechlorination
diazinon	Sethunathan, 73	flooded soil	transformation
2,3-dibromobutane	Castro & Belser, 68	flooded soil	debromination
dibromochloromethane	Bouwer & McCarty, 83b	enriched methanogenic culture	transformation
1,2-dibromo-3-chloropropane	Castro & Belser, 68	flooded soil	dehalogenation
di-*n*-butylphthalate	Benckiser & Ottow, 82	*Pseudomonas pseudoalcaligenes*	transformation
	Shelton & Tiedje, 84a	dilute sewage sludge	transformation
2,5-dichlorobenzoate	Suflita et al., 82	dilute sewage sludge	dechlorination
		dilute sediment	
3,4-dichlorobenzoate	Suflita et al., 82	dilute sewage sludge	dechlorination
		dilute sediment	
3,5-dichlorobenzoate	Horowitz et al., 83	dilute freshwater sediment	dechlorination
	Suflita et al., 82	dilute sewage sludge	deg. to CH_4 & CO_2
		dilute sediment	

Compound	Reference	Microorganism(s)	Degradation/Transformation
1,1-dichloroethene	Barrio-Lage et al., 86	dilute sediment	loss of substrate
cis-dichloroethene	Barrio-Lage et al., 86	dilute sediment	loss of substrate
	Parsons et al., 85	groundwater materials	transformation
	Parsons et al., 84	aquifer material	dechlorination
trans-dichloroethene	Barrio-Lage et al., 86	dilute sediment	transformation
	Parsons et al., 85	groundwater materials	transformation
	Parsons et al., 84	aquifer material	dechlorination
2,3-dichlorophenol	Boyd & Shelton, 84	fresh & acclimated sludge	transformation
2,4-dichlorophenol	Boyd & Shelton, 84	fresh & acclimated sludge	transformation
	Suflita & Miller, 85	aquifer materials	deg. to CH_4 & CO_2
2,5-dichlorophenol	Boyd & Shelton, 84	fresh & acclimated sludge	transformation
	Suflita & Miller, 85	aquifer materials	deg. to CH_4 & CO_2
2,6-dichlorophenol	Boyd & Shelton, 84	fresh & acclimated sludge	transformation
diethylphthalate	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
dihydrodiranillin	Chen et al., 85	rumen fluid	transformation
3-hydroxybenzoate	Taylor et al., 70	Pseudomonas PN-1	mineralization
3,4-dihydroxybenzoic acid	Bakker, 77	enriched nitrifying culture	transformation
2,4-dihydroxyphthalic acid	Taylor & Ribbons, 83	a marine mixed culture	decarboxylation
3,5-dimethoxybenzoic acid	Taylor, 83	Pseudomonas sp. Strain PN-1	demethylation
	Bache & Pfennig, 81	Acetobacterium strains	demethylation
2,6-dimethylphenol	Fedorak & Hrudey, 84	dilute sewage sludge	deg. to CH_4 & CO_2
3,4-dimethylphenol	Fedorak & Hrudey, 84	dilute sewage sludge	deg. to CH_4 & CO_2

Compound	Reference	Microorganism(s)	Degradation/Transformation
3,5-dimethylphenol	Fedorak & Hrudey, 84	dilute sewage sludge	deg. to CH_4 & CO_2
dimethylphthalate	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
di-n-nonylphthalate	O'Grady et al., 85	activated sludge	transformation
ethylacetate	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
ethylene dibromide	Castro & Belser, 68	flooded soil	debromination
	Bouwer & McCarty, 85	enriched methanogenic culture	debromination
ethylene glycol	Dwyer & Tiedje, 83	enriched methanogenic culture	deg. to CH_4 & CO_2
ferulic acid	Taylor, 83	*Pseudomonas* sp. Strain PN-1	demethylation
	Bache & Pfennig, 81	*Acetobacterium* strains	demethylation
	Healy et al., 80	mixed anaerobic cultures	deg. to CH_4 & CO_2
	Healy & Young, 79	acclimated anaerobic mixed culture	deg. to CH_4 & CO_2
	Grbic-Galic, 85	facultative anaerobic bacterium	transformation
	Grbic-Galic & Young, 85	methanogenic consortium	deg. to CH_4 & CO_2
	Frazer & Young, 85	Gram-negative anaerobic bacterium	demethylation
2-fluorobenzoate	Schennen et al., 85	benzoate-degrading denitrifying bacteria	mineralization
3-fluorophthalic acids	Taylor & Ribbons, 83	a marine mixed culture	decarboxylation
gallic acid	Kaiser & Hanselmann, 82	enriched anaerobic culture	deg. to CH_4 & CO_2
	Schink & Pfennig, 82	*Pelobacter acidigallici*	transformation
geraniol	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
guaiacol	Kaiser & Hanselmann, 82	enriched anaerobic culture	deg. to CH_4 & CO_2
	Taylor, 83	*Pseudomonas* sp. strain PN-1	demethylation
hexadecene	Schink, 85	dilute sediment & sludge	deg. to CH_4 & CO_2

Compound	Reference	Microorganism(s)	Degradation/Transformation
4-hydroxyacetanilide	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
hydrocinnamic	Tarvin & Buswell, 34	dilute sewage sludge	deg. to CH_4 & CO_2
2-hydroxybenzoic acid	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
3-hydroxybenzoic acid	Bakker, 77	enriched nitrifying culture	transformation
4-hydroxybenzoic acid	Dutton & Evans, 69	Rhodopseudomonas palustris	mineralization
	Bakker, 77	enriched nitrifying culture	transformation
	Healy & Young, 79	acclimated anaerobic mixed culture	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
4-hydroxybenzyl alcohol	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
3-hydroxybutanone	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
4-hydroxyphthalic acid	Taylor & Ribbons, 83	a marine mixed culture	decarboxylation
indole	Wang et al., 84	enriched methanogenic cultures	deg. to CH_4 & CO_2
2-iodobenzoate	Suflita et al., 82	dilute sewage sludge	deg. to CH_4 & CO_2
		dilute sediment	
3-iodobenzoic acid	Horowitz et al., 83	dilute freshwater sediment	deiodination
	Suflita et al., 82	dilute sewage sludge	deg. to CH_4 & CO_2
		dilute sediment	deg. to CH_4 & CO_2
4-iodobenzoate	Suflita et al., 82	dilute sewage sludge	deg. to CH_4 & CO_2
		dilute sediment	
lignin	Benner & Hodson, 85	thermophilic anaerobic enrichment cultures	deg. to CH_4 & CO_2

Compound	Reference	Microorganism(s)	Degradation/Transformation
lindane	Sethunathan, 73	flooded soil	transformation
	Beland et al., 76	dilute sewage sludge	dechlorination
	Yule et al., 67	flooded soil	dechlorination
	MacRae et al., 69	*Clostridium* sp.	dechlorination
	Jagnow et al., 77	anaerobic bacteria	dechlorination
	Heritage & MacRae, 77a,b	*Clostridium* splenoides	dechlorination
	Tu, 76	anaerobic bacteria	dechlorination
	Mathur & Saha, 75	flooded soil	dechlorination
	Raghu & MacRae, 66	flooded soil	dechlorination
3-methoxycinnamic acid	Bache & Pfennig, 81	*Acetobacterium* strains	demthoxylation
2-methoxyphenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84	dilute sewage sludge	deg. to CH_4 & CO_2
3-methoxyphenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84	dilute sewage sludge	deg. to CH_4 & CO_2
4-methoxyphenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84	dilute sewage sludge	deg. to CH_4 & CO_2
3-methylbutanol	Shelton & Tiedje, 84	dilute sewage sludge	deg. to CH_4 & CO_2
3-o-methylgallic acid	Taylor, 83	*Pseudomonas* sp. Strain PN-1	demethoxylation
methyl parathion	Ou, 85	flooded soil	mineralization
nicotinic acid	Harary, 75	anaerobic bacterium	fermentation
	Imhoff-Stuckle & Pfennig, 83	*Desulfococcus niacini*	mineralization
	Stadtman et al., 72	*Clostridium barkeri*	mineralization
nitrilotriacetic acid	Ward, 85	subsurface soils	mineralization

Compound	Reference	Microorganism(s)	Degradation/Transformation
2-nitrophenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO
3-nitrophenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
4-nitrophenol	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
octadecane	Hambrick, III, et al., 80	dilute sediments	deg. to CH_4 & CO_2
1-octanol	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
2-octanol	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
orange II	Dubin & Wright, 75	*Proteus vulgaris*	reduction
oxalate	Smith & Oremland, 83	dilute aquatic sediments	deg. to CO_2
Ponceau 3R	Dubin & Wright, 75	*Proteus vulgaris*	reduction
Ponceau SX	Dubin & Wright, 75	*Proteus vulgaris*	reduction
parathion	Rajaram & Sethunathan, 75	flooded soil	transformation
	Sethunathan, 75	flooded soil	transformation
pentachlorophenol	Guthrie et al., 84	anaerobic sewage sludge	transformation
phenol	Bakker, 77	enriched nitrifying culture	transformation
	Evans, 77	*Rhodopseudomonas palustris*	
		Pseudomonas strain NP-1	
		methanogenic enriched cultures	deg. to CH_4 & CO_2
	Healy & Young, 78	dilute sewage sludge	deg. to CH_4 & CO_2
	Healy & Young, 79	acclimated anaerobic mixed cultures	deg. to CH_4 & CO_2
	Boyd et al., 83	dilute sewage sludge	deg. to CH_4 & CO_2
	Fedorak & Hrudey, 84	dilute sewage sludge	deg. to CH_4 & CO_2

Compound	Reference	Microorganism(s)	Degradation/Transformation
(phenol, cont)	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
	Barik et al., 85	anaerobic bacterium plus *Wolinella succinogenes*	deg. to CH_4 & CO_2
	Suflita & Miller, 85	groundwater materials	deg. to CH_4 & CO_2
phenol acetic acid	Barik et al., 85	anaerobic bacterium plus *Wolinella succinogenes*	deg. to CH_4 & CO_2
phenolic mixtures	Godsy et al., 83	sewage sludge	deg. to CH_4 & CO_2
1-phenylazo-2-naphthal	Wuhrman et al., 80	*Bacillus cereus*	transformation
β-phenylpropionate	Balba & Evans, 79	benzoate enriched methanogenic culture	deg. to CH_4 & CO_2
phloroglucinol	Tsai & Jones, 75	*Streptococcus bovis*	mineralization
	Evans, 77	*Rhodopseudomonas palustris*	deg. to CH_4 & CO_2
		Pseudomonas strain NP-1 enriched methanogenic cultures	
	Dutton & Evans, 78	*Rhodopseudomonas palustris*	mineralization
	Patel et al., 81	*Caprococcus* sp. Pe, 5	mineralization
	Schink & Pfennig, 82	*Pelobacter acidigallici*	fermentation
	Walker & Taylor, 83	*Fusarium solani*	mineralization
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
m-phthalic acid	Affring et al., 81	dentrifying mixed cultures	mineralization
o-phthalic acid	Affring et al., 81	denitrifying mixed culture	mineralization
	Taylor & Robbins, 83	a marine mixed culture	decarboxylation
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
polyethylene glycol	Dwyer & Tiedje, 83	enriched methanogenic culture	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
propionaldehyde	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2

Compound	Reference	Microorganism(s)	Degradation/Transformation
protocatechuic acid	Dutton & Evans, 69	Rhodopseudomonas palustris	mineralization
	Healy & Young, 79	enriched methanogenic culture	deg. to CH_4 & CO_2
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
pyrogallol	Kaiser & Hanselmann, 82	enriched anaerobic culture	deg. to CH_4 & CO_2
	Schink & Pfennig, 82	Pelobacter acidigallici	fermentation
	Shelton & Tiedje, 84a	dilute sewage sludge	deg. to CH_4 & CO_2
sinapic acid	Bache & Pfennig, 82	Acebacterium woodii	demethylation
Sunset Yellow	Dubin & Wright, 75	Proteus vulgaris	reduction
squaline	Schink, 85	dilute sediment & sludge	deg. to CH_4 & CO_2
syringic acid	Healy & Young, 79	enriched methanogenic culture	deg. to CH_4 & CO_2
	Bache & Pfennig, 81	Acetobacterium woodii	demethylation
	Kaiser & Hanselmann, 82	dilute freshwater sediments	deg. to CH_4 & CO_2
	Taylor, 83	Pseudomonas sp. Strain PN-1	demethylation
	Frazer & Young, 85	gram-neg, anaerobic rods	demethylation
syringol	Kaiser & Hanselmann, 82	enriched anaerobic consortium	deg. to CH_4 & CO_2
syringaldehyde	Healy & Young, 79	enriched methanogenic culture	deg. to CH_4 & CO_2
	Bache & Pfennig, 81	Acetobacterium woodii	demethylation
tartazine	Dubin & Wright, 75	Proteus vulgaris	reduction
tetrachloroquaiacol	Remberger et al., 86	sediment slurries	demethylation
1,1,2,2-tetrachloroethane	Bouwer & McCarty, 83a	enriched methanogenic culture	transformation
tetrachloroethylene	Bouwer et al., 81	dilute sewage sludge	dechlorination
	Bouwer & McCarty, 83a	enriched methanogenic culture	transformation
	Parsons et al., 84	groundwater environment	dechlorination
	Parsons et al., 85	groundwater materials	transformation
	Vogel & McCarty, 85	enriched methanogenic culture	transformation

Compound	Reference	Microorganism(s)	Degradation/Transformation
toluene	Grbic-Galic & Vogel, 86	ferulic acid enriched methanogenic culture	deg. to CH_4 & CO_2
tetrachloroveratrol	Remberger et al., 86	sediment slurries	demethylation
tetrachloroguaiacol	Remberger et al., 86	sediment slurries	demethylation
2,3,6-trichlorobenzoate	Horowitz et al., 83	dilute freshwater sediment	dechlorination deg. to CH_4 & CO_2
	Suflita et al., 82	dilute sewage sludge dilute sediment	deg. to CH_4 & CO_2
3,4,5-trichlorocatechol	Remberger et al., 86	sediment slurries	demethylation
1,1,1-trichloroethane	Bouwer & McCarty, 83a	enriched methanogenic culture	transformation
	Parsons et al., 85	groundwater materials	transformation
	Parsons & Lage, 85	groundwater material	transformation
	Parsons & Lage, 85	aquifer materials	dechlorination
trichloroethylene	Bouwer et al., 81	dilute sewage sludge	dechlorination
	Parsons et al., 84	groundwater environment	dechlorination
	Parsons et al., 85	groundwater materials	transformation
	Kleopfer, 85	flooded soil	transformation
3,4,5-trichloroguaiacol	Remberger et al., 86	dilute sediment	demethylation
2,4,5-trichlorophen-oxyacetic acid	Suflita et al., 84	enriched methanogenic culture	dechlorination
3,4,5-trichloroveratrol	Remberger et al., 86	dilute sediment	demethylation
trifluralin	Parr & Smith, 73	flooded soil	transformation
	Williams & Feil, 71	Rumen bacteria	transformation
	Golab et al., 69	flooded soil	transformation
2,4,6-trihydroxybenzoate	Schink & Pfennig, 82	Pelobacter acidigallici	fermentation

Compound	Reference	Microorganism(s)	Degradation/Transformation
3,4,5-trimethoxybenzoate	Bache & Pfennig, 81	Acetobacterium woodii	demethylation
	Kaiser & Hanselmann, 82	dilute freshwater sediments	deg. to CH_4 & CO_2
	Taylor, 83	Pseudomonas sp. Strain PN-1	demethylation
3,4,5-trimethoxycinnamic acid	Bache & Pfennig, 81	Acetobacterium woodii	demethylation
	Taylor, 83	Pseudomonas sp. Strain PN-1	demethylation
2,4,6-trinitrotoluene	Carpenter et al., 78	Activated sludge system	transformation
	Golab et al., 69	flooded soil	transformation
vanillate	Healy & Young, 79	enriched methanogenic culture	deg. to CH_4 & CO_2
	Bache & Pfennig, 81	Acetobacterium woodii	demethylation
	Kaiser & Hanselmann, 82	enriched anaerobic consortium	deg. to CH_4 & CO_2
	Taylor, 83	Pseudomonas sp. Strain PN-1	demethylation
	Frazer & Young, 85	gram-neg anaerobic rods	demethylation
	Frazer & Young, 86	anaerobic acetogen	deg. to CO_2 & acetate
vanillin	Bache & Pfennig, 81	Acetobacterium woodii	demethylation
veratiate	Taylor, 83	Pseudomonas sp. Strain PN-1	demethylation
m-xylene	Kuhn et al., 85	aquifer material nitrifying conditions	transformation
o-xylene	Grbic-Galic & Vogel, 86	ferulic acid enriched methanogenic culture	deg. to CH_4 & CO_2
p-xylene	Kuhn et al., 85	aquifer material nitrifying conditions	transformation